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(34) Title: VANILLOID RECEPTOR-RELATED NUCLEIC ACIDS AND POLYPEPTIDES

(57) Abstract: This invention provides novel genes and polypeptides of the VR family, identification of tRKA⁺ pain specific genes expressed in DRG, and use of these genes and polypeptides for the treatment of pain and identification of agents useful in the treatment of pain.

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VANILLOID RECEPTOR-RELATED NUCLEIC ACIDS AND POLYPEPTIDES

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CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/297,835 filed on June 13, 2001, U.S. Provisional Application No. 60/351,238, filed on January 22, 2002, U.S. Provisional Application No. 60/352,914, filed on January 29, 2002, U.S. Provisional Application No. 60/357,161, filed on February 12, 2002, U.S.

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Provisional Application No. 60/381,086, filed on May 15, 2002, and U.S. Provisional Application No. 60/381,739, filed on May 16, 2002. These applications are incorporated herein by reference for all purposes.

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BACKGROUND OF THE INVENTION

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Field of the Invention

[0003] This invention pertains to novel vanilloid receptor (VR) related nucleic acids and polypeptides. In particular, the invention relates to proteins that are homologous to known VRs, nucleic acids encoding such proteins, identification of tRKA⁺ pain-specific genes, and the use of these genes and polypeptides in methods of diagnosing pain, methods of identifying compounds useful in treating pain and methods of treating pain.

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Background

[0004] Pain has been defined as the sensory experience perceived by nerve tissue distinct from sensations of touch, pressure, heat and cold. Individuals suffering from pain

typically describe it by such terms as bright, dull, aching, pricking, cutting, burning, etc. This range of sensations, as well as the variation in perception of pain by different individuals, makes a precise definition of pain difficult. Pain as suffering, however, is generally considered to include both the original sensation and the reaction to that sensation.

5 Where pain results from the stimulation of nociceptive receptors and transmitted over intact neural pathways, this is termed nociceptive pain. Alternatively, pain may be caused by damage to neural structures, often manifesting itself as neural supersensitivity, and is referred to as neuropathic pain.

[0005] Neuropathic pain is a particular type of pain that has a complex and variable etiology. It is generally a chronic condition attributable to complete or partial transection of a nerve or trauma to a nerve plexus or soft tissue. This condition is characterized by hyperesthesia (enhanced sensitivity to a natural stimulus), hyperalgesia (abnormal sensitivity to pain), allodynia (widespread tenderness, characterized by hypersensitivity to tactile stimuli) and/or spontaneous burning pain. In humans, neuropathic pain tends to be chronic and debilitating, and occurs during conditions such as trigeminal neuralgia, diabetic neuropathy, post-herpetic neuralgia, late-stage cancer, amputation or physical nerve damage.

[0006] Most drugs including conventional opioids and antidepressants are not practical against chronic pain such as neuropathic pain, either because they are not effective or have serious side effects. For these reasons, alternate therapies for the management of chronic or neuropathic pain are widely sought.

[0007] Stimuli such as heat, cold, stretch, and pressure are detected by specialized sensory neurons within the Dorsal Root Ganglia (DRG). These neurons fire action potentials in response to these mechanical and thermal stimuli, although the molecular mechanism for such detection is not known. Recently, two channels, vanilloid receptor 1 (VR1) and vanilloid receptor-like protein 1 (VRL1), have been isolated from DRG that respond to different thresholds of high heat, and hence act as pain receptors. These channels belong to a family of TRP channels that in *C. elegans* and *D. melanogaster* are involved in mechano- and osmoregulation.

[0008] The VR1 is a calcium channel with six transmembrane domains and a putative pore domain. The channel can be activated by many distinct reagents, including heat, low pH (high proton concentration is present during injury and inflammation), and

capsaicin (the active ingredient in hot chili peppers). The knockout of VR1 in mice has demonstrated that this channel plays a role in pain propagation; however, since the phenotype is rather subtle, it also implies that VR1 is not the sole receptor for high heat and pain. To date, one other homologue of VR1 is known in mammals - the VRL1. VRL1 is structurally very similar to VR1, but is expressed on DRG neurons that are not involved in pain reception (in contrast to VR1).

[0009] The somatic sensory neurons detect external stimuli such as heat, cold and noxious stimuli through the activation of thermal and mechanical receptors/channels. The VR family represents the first example of molecules expressed within the DRG that have such activation capabilities. Since these molecules are relatively specific to sensory neurons (for example, VR1 knockout mice do not have phenotypes outside of pain perception), they represent highly promising targets for developing drugs against pain or other thermal noxious stimuli. VR1 knockout mice have demonstrated that other molecules have to be involved in pain perception. However, despite the large amount of interest generated in the scientific community concerning this class of receptors, so far, no other receptors of this class have been identified.

[0010] In view of the role of the VR members in pain perception, the identification of new members of VR would allow the development of therapeutic candidates specifically designed to block these new TRP channels, which would enable the treatment of various disorders associated with chronic pain. In addition, the identification of new VR members would permit the screening of various drugs to identify those compounds suitable for further, in-depth studies of therapeutic applications.

SUMMARY OF THE INVENTION

[0011] The present invention relates to members of the VR family, in particular TRPV3 (previously known as VRLS, VRLX, VR4 and TRPV7), TRPV4 (previously known as VRL3 and OTRPC4) and TRPM8 (previously known as TRPX) nucleic acids and polypeptides, recombinant materials and methods for their production. In another aspect, the present invention relates to the identification of trkA^+ pain-specific genes expressed in the DRG. In yet another aspect, the present invention relates to methods for using the TRPV3, TRPV4, TRPM8 and trkA^+ pain-specific nucleic acids and polypeptides, including methods for treating pain, inflammation, skin disorders and cancer, methods of diagnosing pain,

inflammation, skin disorders and cancer, methods of identifying agents useful in the treatment of pain, inflammation, skin disorders and cancer and in methods of monitoring the efficacy of a treatment for pain, inflammation, skin disorders and cancer.

TRPV3

- 5 [0012] The invention provides isolated and/or purified TRPV3 nucleic acid molecules, such as: a) a polynucleotide that encodes a mouse TRPV3 protein comprising amino acid residues 1-791 of SEQ ID NO: 2; b) a polynucleotide that encodes a mouse TRPV3 protein comprising amino acid residues 2-791 of SEQ ID NO: 2; c) a polynucleotide that encodes a functional domain of a mouse TRPV3 protein; d) a polynucleotide that encodes a human TRPV3 protein comprising amino acid residues 1-791 of SEQ ID NO 5; e) a polynucleotide that encodes a human TRPV3 protein comprising amino acid residues 2-791 of SEQ ID NO 5; f) a polynucleotide that encodes a functional domain of a human TRPV3 protein; and g) a polynucleotide that is complementary to a polynucleotide of a) through f). In some embodiments, the nucleic acid molecule is a) or b) and comprises a first polynucleotide that is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 3 (mouse TRPV3), or is d) or e) and comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 6 (human TRPV3). The nucleic acids can be 90% or more, or 95% or more, identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 6, or can be identical to the respective polynucleotide. Examples of TRPV3 nucleic acids of the invention include polynucleotides that are 80% or more, 90% or more, or 95% or more, identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 65-2440 of SEQ ID NO: 1 (mouse TRPV3) or nucleotides 57-2432 of SEQ ID NO: 4 (human TRPV3).
- 25 [0013] The invention also provides isolated TRPV3 nucleic acid molecules that encode polypeptides that include one or more functional domains of a mammalian (e.g., human or mouse) TRPV3 polypeptide. The polypeptides encoded by these nucleic acid molecules can include, for example, one or more functional domains such as ankyrin domains, transmembrane regions, pore loop regions, and coiled-coil domains. As an example, the polypeptides can include a pore loop region flanked by two transmembrane regions, and/or four ankyrin domains.

- [0014] Also provided by the invention are isolated and/or purified TRPV3 polypeptides. Such polypeptides include, for example, a) a mouse TRPV3 protein comprising amino acid residues 1-791 of SEQ ID NO: 2; b) a mouse TRPV3 protein comprising amino acid residues 2-791 of SEQ ID NO: 2; c) one or more functional domains of a mouse TRPV3 protein; d) a human TRPV3 protein comprising amino acid residues 1-791 of SEQ ID NO 5; e) a human TRPV3 protein comprising amino acid residues 2-791 of SEQ ID NO 5; and f) one or more functional domains of a human TRPV3 protein. For example, the TRPV3 polypeptides can include one or more functional domains selected from the group consisting of an ankyrin domain, a transmembrane region, a pore loop region, and a coiled-coil domain. In some embodiments, the polypeptides include a pore loop region flanked by two transmembrane regions, and/or four ankyrin domains.
- 10 [0015] Methods for identifying an agent that modulates TRPV3-mediated cation passage through a membrane are also provided by the invention. These methods involve: a) providing a membrane that comprises a TRPV3 polypeptide; b) contacting the membrane with a candidate agent; and c) determining whether passage of one or more cations through the membrane is increased in the presence of the candidate agent compared to passage in the absence of the candidate agent. In some embodiments, the membrane is a cell membrane and cation passage through the membrane is detected by measuring cation influx or efflux across the membrane into or out of the cell. The assay is conducted at a temperature of at least 33°C, in some embodiments. Also provided are methods in which a candidate agent that reduces cation passage is further tested for ability to treat pain by administering the candidate agent to a test animal and determining whether the candidate agent decreases the test animal's response to a pain stimulus. A pain stimulus can include, for example exposure to a temperature above 33°C.
- 25 [0016] The invention also provides methods for reducing pain associated with TRPV3 activity. These methods involve administering to a subject suffering from pain an analgesically effective amount of a compound that reduces TRPV3-mediated cation passage through a membrane or reduces signal transduction from a TRPV3 polypeptide to a DRG neuron. The pain can be with, for example, one or more of heat exposure, inflammation, and tissue damage. Suitable compounds can include, for example, an antibody that specifically binds to a TRPV3 polypeptide, an antisense polynucleotide, ribozyme, or an interfering

RNA that reduces expression of a TRPV3 polypeptide; and/or a chemical compound that reduces cation passage through a membrane that comprises a TRPV3 polypeptide.

[0017] Methods for determining whether pain in a subject is mediated by TRPV3

are also provided by the invention. These methods can involve: obtaining a sample from a region of the subject at which the pain is felt; and testing the sample to determine whether a TRPV3 polypeptide or TRPV3 polynucleotide is present and/or active in the sample. In

some embodiments, the presence of a TRPV3 polypeptide in the sample is detected by determining whether cation passage across membranes of cells in the sample is mediated by a TRPV3 polypeptide. For example, TRPV3 involvement in mediating cation passage across

membranes of the cells can be determined by detecting an increase in cation passage across membranes of the cells when assayed above 33°C compared to cation passage when assayed below 33°C. To distinguish between TRPV3 involvement in mediating cation passage and

involvement by other ion channels (e.g., TRPV1 or TRPV2), the assay can be conducted at a temperature above the activation threshold of TRPV3 but below the activation threshold of the other receptor (e.g., below about 43°C or below about 52°C, respectively, for TRPV1 and TRPV2). As an alternative to assaying for TRPV3-mediated ion channel activity, one can

detect the presence of a TRPV3 polypeptide in the sample by contacting the sample with a reagent that specifically binds to a TRPV3 polypeptide, or detect the presence of a TRPV3 polynucleotide in the sample by contacting nucleic acids from the sample with a test polynucleotide that can hybridize to a TRPV3 polynucleotide.

TRPV4

[0018] The invention also provides isolated TRPV4 nucleic acid molecules. These include, for example, a) a polynucleotide that encodes a mouse TRPV4 protein comprising amino acid residues 1-871 of SEQ ID NO: 14; b) a polynucleotide that encodes a mouse TRPV4 protein comprising amino acid residues 2-871 of SEQ ID NO: 14; c) a

polynucleotide that encodes a polypeptide that comprises one or more functional domains of a mouse TRPV4 protein; d) a polynucleotide that encodes a human TRPV4 protein comprising amino acid residues 1-871 of SEQ ID NO 17; e) a polynucleotide that encodes a human TRPV4 protein comprising amino acid residues 2-871 of SEQ ID NO 17; f) a

polynucleotide that encodes a polypeptide that comprises one or more functional domains of a human TRPV4 protein; and g) a polynucleotide that is complementary to a polynucleotide

of a) through f). In some embodiments, the nucleic acid molecule is a) or b) and comprises a first polynucleotide that is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 15 (mouse TRPV4), or is d) or e) and

comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 18 (human TRPV4). The nucleic acids can

be 90% or more, or 95% or more, identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 15 or SEQ ID NO: 18, or can be identical to the

respective polynucleotide. Examples of TRPV4 nucleic acids of the invention include polynucleotides that are 80% or more, 90% or more, or 95% or more, identical to a second

polynucleotide having a nucleotide sequence as set forth in nucleotides 156-2771 of SEQ ID NO: 13 (mouse TRPV4) or to a nucleotide sequence as set forth in SEQ ID NO: 16 (human TRPV4).

[0019] The invention also provides isolated TRPV4 nucleic acid molecules that

encode polypeptides that include one or more functional domains of a mammalian (e.g.,

human or mouse) TRPV4 polypeptide. The polypeptides encoded by these nucleic acid

molecules can include, for example, one or more functional domains such as ankyrin

domains, transmembrane regions, pore loop regions, and coiled-coil domains. As an

example, the polypeptides can include a pore loop region flanked by two transmembrane regions, and/or three ankyrin domains.

[0020] Also provided by the invention are isolated and/or purified TRPV4

polypeptides. Such polypeptides include, for example, a) a mouse TRPV4 protein

comprising amino acid residues 1-871 of SEQ ID NO: 14; b) a mouse TRPV4 protein

comprising amino acid residues 2-871 of SEQ ID NO: 14; c) one or more functional

domains of a mouse TRPV4 protein; d) a human TRPV4 protein comprising amino acid

residues 1-871 of SEQ ID NO 17; e) a human TRPV4 protein comprising amino acid

residues 2-871 of SEQ ID NO 17; and f) one or more functional domains of a human TRPV4

protein. For example, the TRPV4 polypeptides can include one or more functional domains

selected from the group consisting of an ankyrin domain, a transmembrane region, a pore

loop region, and a coiled-coil domain. In some embodiments, the polypeptides include a

pore loop region flanked by two transmembrane regions, and/or three ankyrin domains.

[0021] Methods for identifying an agent that modulates TRPV4-mediated cation passage through a membrane are also provided by the invention. These methods involve: a)

providing a membrane that comprises a TRPV4 polypeptide; b) contacting the membrane with a candidate agent; and c) determining whether passage of one or more cations through the membrane is increased in the presence of the candidate agent compared to passage in the absence of the candidate agent. Cation influx and/or efflux can be measured as described above for TRPV3. In some embodiments, candidate agents that reduce cation passage are further tested for ability to treat pain by administering the candidate agent to a test animal and determining whether the candidate agent decreases the test animal's response to a pain stimulus.

[0022] Methods for reducing pain associated with TRPV4 activity are provided by the invention. These methods involve administering to a subject suffering from pain an analgesically effective amount of a compound that reduces TRPV4-mediated cation passage through a membrane or reduces signal transduction from a TRPV4 polypeptide to a DRG neuron. The compounds are suitable for treating, for example, neuropathic pain, and can include: a) an antibody that specifically binds to a TRPV4 polypeptide; b) an antisense polynucleotide, ribozyme, or an interfering RNA that reduces expression of a TRPV4 polypeptide; and c) a chemical compound that reduces cation passage through a membrane that comprises a TRPV4 polypeptide.

[0023] The invention also provides methods for determining whether pain in a subject is mediated by TRPV4. These methods involve obtaining a sample from a region of the subject at which the pain is felt, and testing the sample to determine whether a TRPV4 polypeptide or TRPV4 polynucleotide is present and/or active in the sample. The presence and/or activity of the TRPV4 polypeptide can be detected, for example, by determining whether cation passage across membranes of cells in the sample is mediated by a TRPV4 polypeptide, or by contacting the sample with a reagent that specifically binds to a TRPV4 polypeptide. One can detect the presence of a TRPV4 polynucleotide by, for example, contacting nucleic acids from the sample with a test polynucleotide that can hybridize to a TRPV4 polynucleotide.

TRPM8

[0024] Isolated and/or purified TRPM8 nucleic acid molecules are also provided by the invention. These TRPM8 nucleic acid molecules include, for example, a) a polynucleotide that encodes a mouse TRPM8 protein comprising amino acid residues 1-1104

of SEQ ID NO: 8; b) a polynucleotide that encodes a mouse TRPM8 protein comprising amino acid residues 2-1104 of SEQ ID NO: 8; c) a polynucleotide that encodes a polypeptide that comprises one or more functional domains of a mouse TRPM8 protein; d) a polynucleotide that encodes a human TRPM8 protein comprising amino acid residues 1-1268 of SEQ ID NO: 11; e) a polynucleotide that encodes a human TRPM8 protein comprising amino acid residues 2-1268 of SEQ ID NO: 11; f) a polynucleotide that encodes a polypeptide that comprises one or more functional domains of a human TRPM8 protein; and g) a polynucleotide that is complementary to a polynucleotide of a) through f). In some embodiments, the nucleic acid molecule is a) or b) and comprises a first polynucleotide that is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 9 (mouse TRPM8), or is d) or e) and comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 12 (human TRPM8). The nucleic acids can be 90% or more, or 95% or more, identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 9 or SEQ ID NO: 12, or can be identical to the respective polynucleotide. Examples of TRPM8 nucleic acids of the invention include polynucleotides that are 80% or more, 90% or more, or 95% or more, identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 448-3762 of SEQ ID NO: 7 (mouse TRPM8) or nucleotides 61-4821 of SEQ ID NO: 10 (human TRPM8).

[0025] The invention also provides isolated TRPM8 nucleic acid molecules that encode polypeptides that include one or more functional domains of a mammalian (e.g., human or mouse) TRPM8 polypeptide. The polypeptides encoded by these nucleic acid molecules can include, for example, one or more functional domains such as transmembrane regions, pore loop regions, and coiled-coil domains. As an example, the polypeptides can include a pore loop region flanked by two transmembrane regions.

[0026] The invention also provides isolated and/or purified TRPM8 polypeptides. The TRPM8 polypeptides include, for example, a) a mouse TRPM8 protein comprising amino acid residues 1-1104 of SEQ ID NO: 8; b) a mouse TRPM8 protein comprising amino acid residues 2-1104 of SEQ ID NO: 8; c) one or more functional domains of a mouse TRPM8 protein; d) a human TRPM8 protein comprising amino acid residues 1-1268 of SEQ ID NO: 11; e) a human TRPM8 protein comprising amino acid residues 2-1268 of SEQ ID NO: 11; and f) one or more functional domains of a human TRPM8 protein. For example, the

TRPM8 polypeptides can include one or more functional domains selected from the group consisting of a transmembrane region, a pore loop region, and a coiled-coil domain. In some embodiments, the TRPM8 polypeptides of the invention include a pore loop region flanked by two transmembrane regions.

5 [0027] Methods for identifying an agent that modulates TRPM8-mediated cation passage through a membrane are also provided by the invention. These methods involve: a) providing a membrane that comprises a TRPM8 polypeptide; b) contacting the membrane with a candidate agent; and c) determining whether passage of one or more cations through the membrane is increased in the presence of the candidate agent compared to passage in the absence of the candidate agent. In some embodiments, the membrane is a cell membrane and cation passage through the membrane is detected by measuring cation influx or efflux across the membrane into or out of the cell. To identify antagonists that reduce TRPM8-mediated cation passage, the assay typically is conducted under conditions in which TRPM8 allows cation passage in the absence of the antagonist; e.g., at a temperature of about 20°C or less, or in the presence of menthol. Also provided are methods in which a candidate agent that reduces cation passage is further tested for ability to treat pain by administering the candidate agent to a test animal and determining whether the candidate agent decreases the test animal's response to a pain stimulus. A pain stimulus can include, for example exposure to a temperature below 20°C.

20 [0028] In other embodiments, the invention provides methods for identifying an agent that stimulates TRPM8-mediated cation passage through a membrane. These screens for identifying TRPM8 agonists generally are conducted under conditions in which the TRPM8 polypeptides do not mediate cation passage. Such conditions include, for example, temperatures above about 20°C. Agonists of TRPM8-mediated cation passage are useful as flavor enhancers, fragrances, and the like.

25 [0029] The invention also provides methods of reducing pain associated with TRPM8 activity. These methods involve administering to a subject suffering from pain an analgesically effective amount of a compound that reduces TRPM8-mediated cation passage through a membrane or reduces signal transduction from a TRPM8 polypeptide to a DRG neuron. These methods are useful for treating pain that results from, for example, cold exposure, inflammation, tissue damage, and the like. The compounds can be, for example, a) an antibody that specifically binds to a TRPM8 polypeptide; b) an antisense polynucleotide,

ribozyme, or an interfering RNA that reduces expression of a TRPM8 polypeptide; or c) a chemical compound that reduces cation passage through a membrane that comprises a TRPM8 polypeptide.

10 [0030] Methods for determining whether pain in a subject is mediated by TRPM8 are also provided by the invention. These methods involve obtaining a sample from a region of the subject at which the pain is felt; and testing the sample to determine whether a TRPM8 polypeptide or TRPM8 polynucleotide is present and/or active in the sample. In some embodiments, the presence of a TRPM8 polypeptide in the sample is detected by determining whether cation passage across membranes of cells in the sample is mediated by a TRPM8 polypeptide. TRPM8 involvement in mediating cation passage across membranes of the cells can be determined, for example, by detecting an increase or decrease in cation passage across membranes of the cells when assayed below 20°C and/or in the presence of menthol, compared to cation passage when assayed above 20°C and/or in the absence of menthol. Alternatively, or additionally, the presence of a TRPM8 polypeptide in the sample is detected by contacting the sample with a reagent that specifically binds to a TRPM8 polypeptide. The presence of a TRPM8 polynucleotide in the sample can be detected by, for example, contacting nucleic acids from the sample with a test polynucleotide that can hybridize to a TRPM8 polynucleotide.

15 [0031] The invention also provides methods for identifying an agent useful in the modulation of a mammalian sensory response. These methods involve: a) contacting a candidate agent with a test system that comprises a receptor polypeptide selected from the group consisting of TRPM8, TRPV3 and TRPV4; and b) detecting a change in activity of the receptor polypeptide in the presence of the candidate agent as compared to the activity of the receptor polypeptide in the absence of the agent, thereby identifying an agent that modulates receptor activity.

20 [0032] Also provided by the invention are methods for monitoring the efficacy of a treatment of a subject suffering from pain. These methods involve: a) obtaining, at two or more time points in the course of treatment for pain, a sample from a region of the subject at which the pain is felt; and b) testing the samples to determine whether a reduction is observed in amount or activity of one or more members selected from the group consisting of: a TRPV3 polypeptide, a TRPV3 mRNA, a TRPV4 polypeptide, a TRPV4 mRNA, a TRPM8 polypeptide, and a TRPM8 mRNA. In some embodiments, one of the time points is

prior to or simultaneously with administration of the treatment, and the other time point is after treatment has begun.

[0033] The invention provides assays capable of detecting the expression of one or more of TRPV3, TRPV4 or TRPM8 in human tissue. The assays are selected from the group consisting of: a) an assay comprising contacting a human tissue sample with monoclonal antibodies binding to TRPV3, TRPV4 or TRPM8 and determining whether the monoclonal antibodies bind to polypeptides in the sample; and b) an assay comprising contacting a human tissue sample with an oligonucleotide that is capable of hybridizing to a nucleic acid that encodes TRPV3, TRPV4 or TRPM8.

[0034] Methods of treating pain provided by the invention include methods in which a patient suffering from pain mediated by one or more polypeptides selected from the group consisting of TRPV3, TRPV4 and TRPM8 is identified by measuring expression of the polypeptide in tissue from such patient, and administering to such patient an analgesically effective amount of an agent which inhibits the polypeptide.

[0035] The invention also provides methods for identifying an agent useful in the treatment of pain. These methods involve: a) administering a candidate agent to a mammal suffering from pain; b) in a sample obtained from the mammal, detecting an activity or amount of one or more members selected from the group consisting of: a TRPV3 polypeptide, a TRPV3 mRNA, a TRPV4 polypeptide, a TRPV4 mRNA, a TRPM8 polypeptide, and a TRPM8 mRNA; and c) comparing the amount or activity of the member in the presence of the candidate agent with the amount or activity of the member in a sample obtained from the mammal in the absence of the candidate agent, wherein a decrease in amount or activity of the member in the sample in the presence of the candidate agent relative to the amount or activity in the absence of the candidate agent is indicative of an agent useful in the treatment of pain.

[0036] Also provided are methods for identifying an agent that binds to and/or modulates the activity of an mRNA or polypeptide encoded by a TRPV3, TRPV4, or TRPM8 nucleic acid. These methods involve: a) contacting an isolated cell which expresses a heterologous TRPV3, TRPV4, or TRPM8 nucleic acid encoding a polypeptide with the agent; and b) determining binding and/or modulation of the activity of the mRNA or polypeptide by the agent, to identify agents which bind with and/or modulate the activity of the polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] Figures 1A and 1B show differential expression of TRPV3 and TRPV4 genes in the Chung model. Figure 1A: mRNA levels of TRPV3 are increased in a rat model of chronic neuropathic pain. The human cDNA sequence of TRPV3 is used to search the Celera mouse genomic DNA database and two primers are derived from regions that are identical from human and mouse sequences. The primers are used to amplify the rat TRPV3 from total RNA samples from the Chung model (L4 and L5 DRG) and sham-operated animals in a standard reverse-transcriptase polymerase chain reaction (RT-PCR) protocol. The top panel shows the gel image from one RT-PCR experiment and the bottom shows the average fold of regulation of TRPV3 in L4 and L5 DRG neurons from Chung model from three independent experiments. Figure 1B: TRPV4 is up-regulated in a rat model of chronic neuropathic pain. For analysis TRPV4 expression in the Chung model (28- and 50-day), first-strand cDNA equivalent to 30 ng of total RNA is used per reaction and amplified between 32/35 cycles for higher expressing genes and 33/38 cycles for lower-expressing genes. Due to the constraints on the amount of total RNA available, half the volume of the PCR reaction is removed at the lower cycle and the remaining reaction is continued for a further 3 cycles. All the samples are resolved on 4-20% TBE gels and densitometry carried out on the clearest, non-saturated bands.

[0038] Figures 2A-2F show the TRPV3 sequence and genomic localization.

Figure 2A: Rooted tree showing protein sequence relationship of different members of the TRPV ion channel family. Figure 2B: Relative position of TRPV1 (VR1) and TRPV3 coding sequences on mouse (11B4) and human (17p13) chromosomes. Figure 2C: Comparison of mouse TRPV3 protein sequence to other TRPVs (excluding C-terminal half containing transmembrane domains). Identical sequences are highlighted in dark gray, conserved residues, in light gray. Predicted coiled-coil and ankyrin domains are marked and correspond to regions for TRPV3 only. The protein alignment is generated using Megalign and Boxshade at <http://biology.ste.edu/CG/BW.cgi>. The coiled-coil domains are predicted using the program Coils (<http://searchlauncher.bom.bmc.edu/seq-search/struc-predict.html>). The ankyrin domains are predicted using the PFAM protein search (<http://pfam.wustl.edu/limsearch.shtml>). Figure 2D: A schematic of TRPV3 and predicted membrane topology. Figure 2E: Kyte Doolittle hydrophobicity plot of TRPV3 sequences showing the 6 transmembrane domains (1-6) and the pore domain (P). Figure 2F: Coiled-

coil domain prediction of TRPV3 sequence by Coils shows two 14-mer peaks at the N-terminal, prior to ankyrin sequences.

[0039] Figures 3A-3D demonstrate that TRPV3 is activated by heat. Currents evoked by heat in TRPV3 expressing Chinese Hamster Ovary (CHO) cells. Figure 3A: Inward current to temperature ramp, $V_h = -60$ mV, in calcium free external solutions. Figure 3B: Heat evoked currents of the same cell in Ca^{2+} -free and subsequently in Ca^{2+} containing solutions showing increased inward current in Ca^{2+} conditions. Figure 3C: Semi-logarithmic plot of current against temperature with double exponential fitted line for the same trace as Figure 3A. Note the discontinuity at $\sim 32^\circ\text{C}$ (arrow). Figure 3D: Current-voltage relationship in calcium containing external solution showing the pronounced outward rectification of TRPV3 at 48°C but not at room temperature. Note the small outward currents at room temperature.

[0040] Figures 4A-4D. TRPV3 becomes sensitized to repeated applications of the heat stimulus. Figure 4A: Repeated heat steps from 25 – 45°C evoke increased inward current responses. Figure 4B: The outward rectification becomes more pronounced with repeated voltage ramps in 48°C external solution. Both experiments are conducted in the presence of 2 mM $CaCl_2$ in the external solution. Figure 4C: Control protocol for antagonist experiments. Note that the responses continue to sensitize with repeated heat steps in the absence of putative antagonists. Figure 4D: 1 μM ruthenium red attenuates the sensitization and inhibits the heat response.

[0041] Figure 5. TRP Channels in thermosensation. Four TRP channels implicated in thermosensation cover most but not all physiologically relevant temperatures.

[0042] Figures 6A-6D show results of an analysis of the nucleotide and amino acid sequences of TRPM8. Figure 6A: Comparison of mouse TRPM8 protein sequence to some of its closest relatives, TRPM1 (human Melastatin, GI 6006023), TRPM2 (human, GI 4507688) and TRPM7 (mouse Chak, GI 14211382). The alignment is generated using Megalign and Boxshade. Identical or conserved residues are shown in white letters on a black background. Figure 6B: Phylogenetic tree showing protein sequence relationship of different members of the TRP ion channel super-family. TRPs are subdivided into three main subfamilies: TRPMs, TRPVs and TRPCs. The TRPMs do not contain any Ankyrin domains in their N-terminal domains. The transmembrane domains have the highest homology among different classes of TRP channels. Figure 6C: Kyte Doolittle

hydrophobicity plot of TRPM8 sequences showing the eight hydrophobic peaks demarking the potential transmembrane regions of the protein that spans from 695-1024 amino acids. Figure 6D: Coiled-coil domain prediction of TRPM8 sequence by the program coils shows multiple 14-mer peaks at the N- and C-terminus of the transmembrane spanning domains (<http://searchlauncher.bcm.tmc.edu/seq-search/struc-predict.html>).

[0043] Figures 7A-7E: Increase in intracellular calcium concentration ($[Ca^{2+}]_i$) in TRPM8-expressing CHO cells in response to cold and menthol. Figure 7A: mTRPM8 CHO cells show a rapid increase in $[Ca^{2+}]_i$ when the temperature reaches $\sim 15^\circ\text{C}$. Non-transfected CHO cells do not show a response to cold. Removal of external Ca^{2+} completely abolishes the response to cooling. Figure 7B: The estimated average threshold temperature at which $[Ca^{2+}]_i$ begins to increase is approximately 23°C for mTRPM8. TRPM8-expressing CHO cells are cooled from 33 – 23°C , upon which an increase in Ca^{2+} is observed. Continuous cooling of the cells to 20°C shows a marked Ca^{2+} increase followed by a rapid return to near-basal levels upon warming to 33°C . Figure 7C: TRPM8 responses, evoked by repeated applications of a 23°C temperature stimulus show little desensitization in calcium-containing standard bath solution. Figure 7D: TRPM8 responds to menthol at 25°C . Intensity of the TRPM8 response is dependent on menthol concentrations. A 10-fold increase in menthol concentration results in a larger influx of Ca^{2+} . This response is suppressed in the absence of extracellular Ca^{2+} . Non-transfected CHO cells exhibit no increase in $[Ca^{2+}]_i$ upon application of menthol. Figure 7E: At 33°C , 10 μM menthol does not elicit an influx of Ca^{2+} . When the temperature of the bath solution is lowered to 30°C , a marked increase in intracellular Ca^{2+} is observed. Additionally, repeated applications of menthol do not appear to desensitize TRPM8-expressing cells. These experiments suggest that menthol simulates the effect of cooling in TRPM8-expressing cells. This identification of a cold-sensing TRP channel involved in thermoreception reveals an expanded role for this family in somatic sensory detection.

[0044] Figures 8A-8B show an increase in intracellular calcium concentration $[Ca^{2+}]_i$ in TRPM8-expressing CHO cells in response to cold. Figure 8A: TRPM8-transfected CHO cells show a rapid increase in $[Ca^{2+}]_i$ when the temperature is lowered from 25°C to 15°C . The stimulus period is indicated below the traces. Non-transfected CHO cells do not show a response to cold. Removal of external Ca^{2+} completely suppresses the response to cooling. Experiments are performed in triplicate. The average response (\pm SEM) of 20-30

cells from a representative experiment is presented. Figure 8B: Increase in $[Ca^{2+}]_i$ due to decrease in temperature from 35°C to 13°C in TRPM8⁺ cells. The panel shows mean \pm SEM for 34 individual cells. Note the increase starts to occur between 22°C and 25°C.

[0045] Figures 9A-9B show that current is evoked by reduction in temperature in TRPM8-expressing CHO cells. Figure 9A: Outward currents evoked at +60 mV by reducing the temperature from 35°C to 10°C. In this cell the current activates at 19.3°C as indicated in the right hand panel. Figure 9B: Current-voltage relationship for currents activated at 20.5°C and 33.5°C. Increasing the temperature reduces the amplitude of outward currents.

[0046] Figures 10A-10B show that current is evoked by menthol in TRPM8-expressing CHO cells. Figure 10A: Inward currents evoked by 1 mM menthol ($V_h = -60$ mV) are inactivated by increasing the temperature from 25°C to 45°C. Figure 10B: Current-voltage relationship for response to 1 mM menthol. Currents show pronounced outward-rectification in the presence of menthol not seen in the absence of this agonist.

[0047] Figures 11A-11B show a dose-response curve for menthol-stimulated current in TRPM8-expressing CHO cells. The voltage employed was +60 mV. Figure 11A: Single examples, from two different cells, of current evoked by applying 0.1, 0.5, 1 and 10 mM menthol at 22°C and 35°C. Figure 11B: Comparison of response (mean \pm SEM, $n=5$ for all points) of current evoked by menthol either at 22°C or 35°C.

DESCRIPTION OF THE SEQUENCE LISTING

[0048] SEQ ID NO: 1 provides a nucleotide sequence that encodes a mouse TRPV3 polypeptide, and upstream and downstream regions. The open-reading frame extends from nucleotides 65-2440.

[0049] SEQ ID NO: 2 provides an amino acid sequence of a mouse TRPV3 polypeptide.

[0050] SEQ ID NO: 3 provides nucleotide sequences for all polynucleotides that code for the mouse TRPV3 amino acid sequence presented in SEQ ID NO: 2.

[0051] SEQ ID NO: 4 provides a nucleotide sequence that encodes a human TRPV3 polypeptide, and an upstream non-coding region. The open-reading frame extends from nucleotides 57-2432.

[0052] SEQ ID NO: 5 provides an amino acid sequence of a human TRPV3 polypeptide.

[0053] SEQ ID NO: 6 provides nucleotide sequences for all polynucleotides that code for the human TRPV3 amino acid sequence presented in SEQ ID NO: 5.

[0054] SEQ ID NO: 7 provides a nucleotide sequence that encodes a mouse TRPM8 polypeptide, and upstream and downstream non-coding regions. The coding region extends from nucleotides 448-3762.

[0055] SEQ ID NO: 8 provides an amino acid sequence of a mouse TRPM8 polypeptide.

[0056] SEQ ID NO: 9 provides nucleotide sequences for all polynucleotides that code for the mouse TRPM8 amino acid sequence presented in SEQ ID NO: 8.

[0057] SEQ ID NO: 10 provides a nucleotide sequence that encodes a human TRPM8 polypeptide, and upstream and downstream non-coding regions. The coding region extends from nucleotides 61-4821.

[0058] SEQ ID NO: 11 provides an amino acid sequence of a human TRPM8 polypeptide.

[0059] SEQ ID NO: 12 provides nucleotide sequences for all polynucleotides that code for the human TRPM8 amino acid sequence presented in SEQ ID NO: 11.

[0060] SEQ ID NO: 13 provides a nucleotide sequence that encodes a mouse TRPV4 polypeptide, and upstream and downstream regions. The open-reading frame extends from nucleotides 156-2771.

[0061] SEQ ID NO: 14 provides an amino acid sequence of a mouse TRPV4 polypeptide.

[0062] SEQ ID NO: 15 provides nucleotide sequences for all polynucleotides that code for the mouse TRPV4 amino acid sequence presented in SEQ ID NO: 14.

[0063] SEQ ID NO: 16 provides a nucleotide sequence that encodes a human TRPV4 polypeptide.

[0064] SEQ ID NO: 17 provides an amino acid sequence of a human TRPV4 polypeptide.

[0065] SEQ ID NO: 18 provides nucleotide sequences for all polynucleotides that code for the human TRPV4 amino acid sequence presented in SEQ ID NO: 17.

DETAILED DESCRIPTION

Definitions

[0066] A "host cell," as used herein, refers to a prokaryotic or eukaryotic cell that contains heterologous DNA that has been introduced into the cell by any means, e.g., electroporation, calcium phosphate precipitation, microinjection, transformation, viral infection and the like.

[0067] "Heterologous" as used herein means "of different natural origin" or represent a non-natural state. For example, if a host cell is transformed with a DNA or gene derived from another organism, particularly from another species, that gene is heterologous with respect to that host cell and also with respect to descendants of the host cell which carry that gene. Similarly, heterologous refers to a nucleotide sequence derived from and inserted into the same natural, original cell type, but which is present in a non-natural state, e.g., a different copy number, or under the control of different regulatory elements.

[0068] A "vector" molecule is a nucleic acid molecule into which heterologous nucleic acid may be inserted which can then be introduced into an appropriate host cell.

Vectors preferably have one or more origins of replication, and one or more sites into which the recombinant DNA can be inserted. Vectors often have convenient means by which cells with vectors can be selected from those without, e.g., they encode drug resistance genes.

Common vectors include plasmids, viral genomes, and (primarily in yeast and bacteria) "artificial chromosomes".

[0069] "Plasmids" generally are designated herein by a lower case p preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art. Starting plasmids disclosed herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids by routine application of well-known, published procedures. Many plasmids and other cloning and expression vectors that can be used in accordance with the present invention are well-known and readily available to those of skill in the art. Moreover, those of skill readily may construct any number of other plasmids suitable for use in the invention. The properties, construction and use of such plasmids, as well as other vectors, in the present invention will be readily apparent to those of skill from the present disclosure.

[0070] The terms "nucleic acid", "DNA sequence" or "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in manner similar to naturally-occurring nucleotides. Although polynucleotide sequences presented herein recite "T" (for thymidine), which is found only in DNA, the sequences also encompass the corresponding RNA molecules in which each "T" in the DNA sequence is replaced by "U" for uridine.

[0071] The term "isolated" refers to material that is substantially or essentially free from components which normally accompany the material as found in its native state. Thus, the polypeptides and nucleic acids of the invention do not include materials normally associated with their *in situ* environment. An isolated nucleic acid, for example, is not associated with all or part of the chromosomal DNA that would otherwise flank the nucleic acid. Typically, isolated proteins of the invention are at least about 80% pure, usually at least about 90%, and preferably at least about 95% pure as measured by band intensity on a silver stained gel or other method for determining purity. Protein purity or homogeneity can be indicated by a number of means well-known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualization upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized.

[0072] The terms "identical" or percent "identity", in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

[0073] The phrase "substantially identical", in the context of two nucleic acids or polypeptides, refers to two or more sequences or subsequences that have at least 70%, preferably 80%, most preferably 90-95% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably the sequences are

substantially identical over at least about 150 residues. In a most preferred embodiment, the sequences are substantially identical over the entire length of the coding regions.

[0074] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0075] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.*, 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.*, 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA*, 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and ITASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), or by visual inspection (see generally, *Current Protocols in Molecular Biology*, F.M. Ausubel et al., eds, Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

[0076] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., *J. Mol. Biol.*, 215:403-410 (1990) and Altschul et al., *Nucleic Acids Res.*, 25:3389-3402 (1997), respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high-scoring sequence pairs (HSPs) by identifying short words of length *W* in the query sequence, which either match or satisfy some positive-valued threshold score *T* when aligned with a word of the same length in a database sequence. *T* is referred to as the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters *M* (reward score for a pair of matching residues, always > 0) and *N* (penalty score for

mis-matching residues, always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity *X* from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more

negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters wordlength (*W*), *T* and *X* determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a *W* of 11, an expectation (*E*) of 10, *M*=5, *N*=4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a *W* of 3, an *E* of 10 and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA*, 89:10915 (1989)). Percent identities, where specified herein, are typically calculated using the Blast 2.0 implementation using the default parameters.

[0077] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Natl. Acad. Sci. USA*, 90:5873-5877 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (*P*(*N*)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0078] Another indication that two polynucleotides are substantially identical is that the polynucleotides hybridize to each other under specified hybridization conditions. Examples of stringent hybridization conditions include: incubation temperatures of about 25°C to about 37°C; hybridization buffer concentrations of about 6 x SSC to about 10 x SSC; formamide concentrations of about 0% to about 25%; and wash solutions of about 6 x SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40°C to about 50°C; buffer concentrations of about 9 x SSC to about 2 x SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5 x SSC to about 2 x SSC. Examples of high stringency conditions include: incubation temperatures of about 55°C to about 68°C; buffer concentrations of about 1 x SSC to about 0.1 x SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1 x SSC,

0.1 x SSC or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2 or more washing steps, and wash incubation times are about 1, 2 or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

5 [0079] A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross-reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions.

10 Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described below.

[0080] "Conservatively modified variations" of a particular polynucleotide sequence refers to those polynucleotides that encode identical or essentially identical amino acid sequences, or where the polynucleotide does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of "conservatively modified variations". Every polynucleotide sequence described herein which encodes a polypeptide also describes every possible silent variation, except where otherwise noted. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each "silent variation" of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0081] Furthermore, one of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations" where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art (see, e.g., Creighton, *Proteins*,

W.H. Freeman and Company (1984)). Individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids in an encoded sequence are also "conservatively modified variations".

[0082] The term "recombinant" when used with reference to a cell, or nucleic acid, or vector, indicates that the cell, or nucleic acid, or vector, has been modified by the introduction of a heterologous nucleic acid or the alteration of a native nucleic acid, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells can contain genes that are not found within the native (non-recombinant) form of the cell or can express native genes that are otherwise abnormally expressed, under expressed or not expressed at all. Recombinant cells can also contain genes found in the native form of the cell wherein the genes are modified and re-introduced into the cell by artificial means. The term also encompasses cells that contain a nucleic acid endogenous to the cell that has been modified without removing the nucleic acid from the cell; such modifications include those obtained by gene replacement, site-specific mutation and related techniques.

[0083] The term "modulate" refers to a change in the activity and/or amount of TRPV3, TRPV4 or TRPM8 proteins. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional or immunological properties of such proteins. The term "modulation" also refers to a change in the increase or decrease in the level of expression of mRNA or protein encoded by the TRPV3, TRPV4, and TRPM8 genes.

[0084] The term "operably-linked", as used herein, refer to functionally-related nucleic acid sequences. A promoter is operably associated or operably-linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably-linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

[0085] The term "agonist", as used herein, refers to a molecule which, when bound to the TRPV3, TRPV4 and TRPM8 proteins, increases or prolongs the duration of the effect of the biological or immunological activity of such proteins. Agonists may include proteins, nucleic acids, carbohydrates or any other molecules which bind to and modulate the effect of these proteins.

[0086] The term "antagonist", as used herein, refers to a molecule which, when bound to TRPV3, TRPV4 and TRPM8 proteins, decreases the amount or the duration of the effect of the biological or immunological activity of these proteins. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies or any other molecules which decrease the effect of these proteins. The term "antagonist" can also refer to a molecule which decreases the level of expression of mRNA and/or translation of protein encoded by TRPV3, TRPV4, and TRPM8 genes. Examples of such antagonists include antisense polynucleotides, ribozymes and double-stranded RNAs.

[0087] In practicing the present invention, many conventional techniques in molecular biology, microbiology and recombinant DNA are used. These techniques are well-known and are explained in, e.g., *Current Protocols in Molecular Biology*, Vols. I, II and III, F.M. Ausubel, ed. (1997); Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (2001); *DNA Cloning: A Practical Approach*, Vols. I and II, D.N. Glover, ed. (1985); *Oligonucleotide Synthesis*, M.L. Gait, ed. (1984); *Nucleic Acid Hybridization*, Hames and Higgins (1985); *Transcription and Translation*, Hames and Higgins, eds. (1984); *Animal Cell Culture*, R.I. Freshney, ed. (1986); *Immobilized Cells and Enzymes*, IRL Press (1986); Perbal, *A Practical Guide to Molecular Cloning*, the series, *Methods in Enzymology*, Academic Press, Inc. (1984); *Gene Transfer Vectors for Mammalian Cells*, J.H. Miller and M.P. Calos, eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1987); and *Methods in Enzymology*, Vols. 154 and 155, Wu and Grossman, and Wu, eds., respectively.

Description of the Preferred Embodiments

[0088] The present invention relates to novel nucleic acids known as TRPV3 (previously known as VR_{LX}, VR_L-S, VR4 and TRPV7), TRPV4 (previously known as VR_{L3} and OTRPC4), and TRPM8 (previously known as TRPX) that are homologous to the VR_{L1} polypeptides encoded by these nucleic acids, recombinant materials and methods for their production. The specific names given to the three genes follow the nomenclature suggested in Montell et al., *Molecular Cell*, 9:229-231 (2002). The genes have been found to be expressed either in keratinocytes or the DRG, and both TRPV3 and TRPM8 proteins function in temperature sensation. In addition, expression of the TRPV3 and TRPV4 genes

is up-regulated in a rat injury model (see Examples 4 and 6). The present invention also relates to the identification of trkA^+ pain-specific genes that are expressed in the DRG. Since the aforementioned genes are expressed in keratinocytes and the DRG, function in temperature sensation, and are up-regulated in response to injury, these genes and their related polypeptides can serve as specific therapeutic targets for the design of drugs to treat chronic and nociceptive pain, inflammation and skin disorders. Accordingly, the invention also relates to methods for identifying agents useful in treating pain, inflammation and skin disorders, methods for treating pain, inflammation and skin disorders and methods of monitoring the efficacy of a treatment, utilizing these genes and polypeptides. These genes and related polypeptides can also be utilized in diagnostic methods for the detection of pain, inflammation and skin disorders.

[0089] TRPV3, TRPV4 and TRPM8 belong to the VR family. A Hidden Markov Model (HMM) of the VR_{L1} and VR_{L3} proteins from different mammalian species including human and an HMM model against Transmembrane 6 (TM6) domain of all known TRP/VRs has been constructed. The six-frame translation of the Human Celera database has been searched against the VR model. Multiple new putative exons with high homology (70% identical and 82% similar in conserved regions among the different VR/TRPs) to Transmembrane 4 (TM4) and TM6 domains to the known TRPs have been identified. These exons map to bacterial artificial chromosomes containing specific human sequences from the High Throughput Genome Sequence (HTGS) database. All the newly-identified exons belong to three new genes of the VR family. Subsequently, RT-PCR has confirmed that these genes are expressed in the DRG or keratinocytes. The structural homology to known TRP channels, the genes' expression in DRG or keratinocytes, their function as temperature-sensitive channels, and the up-regulation of TRPV3 and TRPV4 gene expression observed in a rat injury model in the DRG, indicate that the new genes act as important sensory receptors.

TRPV3: An Ion Channel Responsive to Warm and Hot Temperatures

[0090] TRPV3 is the first molecule described to be activated at warm and hot temperatures, and to be expressed in skin cells (see Examples 2 and 3). TRPV3 signaling mediates a cell-autonomous response in keratinocytes upon exposure to heat. The heat-induced TRPV3 signal is transferred to nearby free nerve endings, thereby contributing to

conscious sensations of warm and hot. This is supported by indirect evidence that skin cells can act as thermal receptors. For instance, while dissociated DRG neurons can be directly activated by heat and cold, warm receptors have only been demonstrated in experiments where skin-nerve connectivity is intact (see Hensel et al., *Pfugers Arch.*, 329:1-8 (1971), Hensel et al., *J. Physio.*, 204:99-112 (1969)). TRPV3 has an activation threshold around 33-35°C. The presence of such a warm receptor in skin (with a resting temperature of 34°C) and not DRG neurons (with a resting temperature of 37°C at the cell body) prevents a warm-channel like TRPV3 from being constitutively active at core 37°C temperatures. The residual heat sensitivity in TRPV1 knockout mice also involves skin cells: while dissociated DRG neurons from TRPV1-null animals do not respond to moderate noxious stimulus at all, skin-nerve preparations from such animals do respond (see Caterina et al., *Science*, 288:306-313 (2000); Davis et al., *Nature*, 405:183-187 (2000); Roza et al., Paper presented at the 31st Annual meeting for the Society of Neuroscience, San Diego, CA (2001)). Collectively these data indicate that a warm/heat receptor is present in the skin, in addition to the heat receptors in DRGs. While synapses have not been found between keratinocytes and sensory termini; ultrastructural studies have shown that keratinocytes contact, and often surround, DRG nerve fibers through membrane-membrane apposition (see Hilliges et al., *J. Invest. Dermatol.*, 104:134-137 (1995) and Cauna, *J. Anat.*, 115:277-288 (1973)). Therefore, heat-activated TRPV3 signal from keratinocytes can be transduced to DRG neurons through direct chemical signaling. One potential signaling mechanism can involve ATP. P2X3, an ATP-gated channel, is present in sensory endings, and analysis of P2X3 knockout mice show a strong deficit in coding of warm temperatures (see Souslova et al., *Nature*, 407:1015-1017 (2000); Cockayne et al., *Nature*, 407:1011-1015 (2000)). Furthermore, release of ATP from damaged keratinocytes has been shown to cause action potentials in nociceptors via the P2X receptors (see Cook et al., *Pain*, 95:41-47 (2002)). Since TRPV3 is activated at innocuous warm and noxious hot temperatures and is expressed in skin, this gene can serve as a therapeutic target for the design of drugs useful in treating pain, inflammation and skin disorders, e.g., those associated with sunburn and other sensitized states.

[0091] In one aspect, the invention provides isolated nucleic acids encoding a mammalian TRPV3 protein. These include an isolated and/or recombinant nucleic acid molecule that encodes a mouse TRPV3 protein having an amino acid sequence as shown in SEQ ID NO: 2. For example, the TRPV3-encoding nucleic acids of the invention include

those that have a nucleotide sequence as set forth in SEQ ID NO: 1, from nucleotides 65-2440. The nucleic acids of the invention can include not only the coding region, but also the non-coding regions that are upstream and downstream of the coding region and also are provided in SEQ ID NO: 1. The invention also provides an isolated mouse TRPV3 polypeptide having an amino acid sequence as shown in SEQ ID NO: 2. Also provided are numerous other nucleic acids that encode this mouse TRPV3 polypeptide; the nucleotide sequences of these nucleic acids are shown in SEQ ID NO: 3.

[0092] Human TRPV3 polypeptides and polynucleotides are also provided by the invention. For example, the invention provides an isolated and/or recombinant human TRPV3-encoding polynucleotide encoding a human TRPV3 polypeptide having an amino acid sequence as set forth in SEQ ID NO: 5. These nucleic acid molecules include those that have a nucleotide sequence as set forth in nucleotides 57-2432 of SEQ ID NO: 4. Upstream and downstream non-coding regions are also provided in SEQ ID NO: 4. Also provided by the invention are isolated human TRPV3 polypeptides having an amino acid sequence as set forth in SEQ ID NO: 5. The invention also provides numerous other nucleic acids that encode this human TRPV3 polypeptide; the nucleotide sequences of these nucleic acids are shown in SEQ ID NO: 6.

TRPV4: An Ion Channel that is Activated by Pain

[0093] TRPV4 is a TRP channel protein that is expressed in adult mouse kidney, newborn dorsal root ganglion and adult trigeminal tissue (see Example 5). TRPV4 is a nonselective cation channel that is activated by decreases in, and is inhibited by increases in, extracellular osmolarity indicating that this channel functions as an osmosensor channel (see, e.g., Strothmann et al., *Nat. Cell Biol.*, 2:695-702 (2000)). In addition, expression of the TRPV4 gene is up-regulated in a rat injury model (see Example 6). Accordingly, the TRPV4 gene can serve as a therapeutic target for the design of drugs to treat pain, kidney disorders and migraine.

[0094] The invention provides isolated nucleic acids that encode a mammalian TRPV4 protein. These include the isolated and/or recombinant nucleic acid molecule that encodes mouse TRPV4 protein having an amino acid sequence as set forth in SEQ ID NO: 14. Included among these nucleic acid molecules are those that have a nucleotide sequence as set forth in nucleotides 156-2771 of SEQ ID NO: 13. Upstream and downstream non-

coding sequences are also provided. Also provided by the invention are isolated mouse TRPV4 polypeptides having an amino acid sequence as set forth in SEQ ID NO: 14.

Numerous other nucleic acids that encode this mouse TRPV4 polypeptide are also provided by the invention. The nucleotide sequences of such nucleic acids are shown in SEQ ID NO:

5 15.

[0095] The mammalian TRPV4-encoding nucleic acids also include the isolated and/or recombinant nucleic acid molecules that encode human TRPV4 protein that has an amino acid sequence as set forth in SEQ ID NO: 17. Such nucleic acid molecules include those having a nucleotide sequence as set forth in SEQ ID NO: 16. Also provided are isolated human TRPV4 polypeptides having an amino acid sequence as set forth in SEQ ID NO: 17. The invention also provides numerous other nucleic acids that encode this human TRPV4 polypeptide; the nucleotide sequences of these nucleic acids are shown in SEQ ID NO: 18.

TRPM8: An Ion Channel Responsive to Cold Temperatures and to Menthol

[0096] TRPM8 is activated by cold stimuli and a cooling agent (menthol) and is expressed in a select group of DRG neurons that share characteristics of thermoreceptive neurons (see Examples 8 and 9).

[0097] Cells over-expressing TRPM8 show increased intracellular calcium levels when subjected to cold temperatures ranging from 23°C to 10°C (the lower limit of our temperature-controlled perfusion system). The calcium influx and electrophysiological studies described below demonstrate that TRPM8 is a non-selective, plasma membrane cation channel activated by cold temperatures. The ionic permeability of TRPM8 is similar to that of other TRP channels, which are permeable to both monovalent and divalent cations, although calcium permeability estimates (P_{Ca}/P_{Na}) vary from 0.3 to 1.4 (see, e.g., Harteneck et al., *Trends Neurosci.*, 23:159-166 (2000)). Menthol is a cooling compound that likely acts on endogenous cold-sensitive channel(s) (see Schafer et al., *J. Gen. Physiol.*, 88:757-776 (1986)). That TRPM8-expressing cells are activated and modulated by menthol reinforces the idea that TRPM8 indeed functions as a cold-sensitive channel *in vivo*. The finding that the sensitivity to menthol is dependent on temperature is consistent with the behavior of a subset of isolated DRG neurons that show a raised 'cold' threshold in the presence of menthol (see Reid and Florida, *Nature*, 413:480 (2001)). With respect to the mechanism of

TRPM8 activation, TRPM8 could be directly gated by cold stimulus through a conformational change, or cold temperatures could act through a second messenger system that in turn activates TRPM8. The rapid activation by menthol suggests a direct gating mechanism, at least for this mode of activation.

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[0098] The expression pattern observed for TRPM8 is consistent with a role in cold thermoreception. First, TRPM8 mRNA is highly-specific to DRG neurons. Within the DRG, TRPM8 is expressed in the small-diameter non-myelinated neurons, which correspond to the c-fiber thermoreceptor and nociceptors (see Scott, *Sensory Neurons: Diversity, Development and Plasticity*, Oxford University Press, NY (1992)). The lack of TRPM8 expression in tRKA knockout mice, whose DRGs lack all thermoreceptor and nociceptive neurons, corroborates this finding. Furthermore, the lack of co-expression with VR1, CGRP or IB4 in the adult suggests that TRPM8 is expressed in a unique population of DRG neurons distinct from well-characterized heat nociceptors. Both soma size of neurons that express VR1 (medium-large neurons) and their co-expression with NF200 (80% co-expression (see Caterina et al., *Nature*, 398:436-441 (1999))) strongly argues that cells expressing TRPM8 and VR1 are also distinct. Therefore, by using various markers it is shown below that TRPM8 is expressed in a sub-class of nociceptors/thermoreceptors that is distinct from noxious heat sensing neurons, and this correlates well with physiological studies of cold-sensitive DRG neurons (see Hensel, *Thermoreception and Temperature Regulation*, Academic Press, London (1981)). A human gene with a high degree of similarity to mouse TRPM8 but no known function was recently shown to be expressed in prostate tissue (see Tsavalier et al., *Cancer Res.*, 61:3760-3769 (2001)).

[0099] As the first molecule to respond to cold temperatures and menthol, TRPM8 offers interesting insight into the fundamental biology of cold perception. Modulation of TRPM8 activity is also relevant for therapeutic applications: cold treatment is often used as a method of pain relief, and in some instances, hypersensitivity to cold can lead to cold allodynia in patients suffering from neuropathic pain. Modulation of TRPM8 activity is also relevant for treating acute pain, e.g., toothache and other trigeminal focused pain; and for treating cancer, particularly prostate cancer and other prostate disorders.

[0100] The invention provides isolated nucleic acids encoding a TRPM8 mammalian protein. These include the isolated and/or recombinant nucleic acid molecules that encode mouse TRPM8 protein that have an amino acid sequence as set forth in SEQ ID

NO: 8. For example, the invention provides recombinant and/or isolated nucleic acid molecules that have a nucleotide sequence as set forth in nucleotides 448-3762 of SEQ ID NO: 7. Upstream and downstream non-coding regions are also provided. The invention also provides isolated mouse TRPM8 polypeptides that include an amino acid sequence as set forth in SEQ ID NO: 8. Also provided are numerous other nucleic acids that encode this mouse TRPM8 polypeptide. Nucleotide sequences of these nucleic acids are provided in SEQ ID NO: 9.

[0101] The nucleic acids encoding a mammalian TRPM8 protein also include isolated and/or recombinant nucleic acid molecules that encode a human TRPM8 protein comprising an amino acid sequence as set forth in SEQ ID NO: 11. For example, the invention provides an isolated and/or recombinant nucleic acid molecule that includes a nucleotide sequence as set forth from nucleotides 61-4821 of SEQ ID NO: 10. Upstream and downstream non-coding regions are also provided by the invention. The invention also provides isolated human TRPM8 polypeptides having an amino acid sequence as set forth in SEQ ID NO: 11. The TRPM8 protein is responsive to cold and menthol.

Nucleic Acid Molecules

[0102] Nucleic acid molecules of the present invention also include isolated nucleic acid molecules that have at least 80% sequence identity, preferably at least 90% identity, preferably at least 95% identity, more preferably at least 98% identity, and most preferably at least 99% identity to a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 14 or SEQ ID NO: 17, respectively, over the entire coding region or over a subsequence thereof. Such nucleic acid molecules include a nucleic acid having a nucleotide sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 18, as set forth above.

[0103] Nucleic acids of the present invention include isolated nucleic acid molecules encoding polypeptide variants which comprise the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 14 or SEQ ID NO: 17, respectively. Nucleic acids that are amplified using a primer pair disclosed herein are also encompassed by the present invention.

[0104] Further nucleic acids of the present invention also include fragments of the aforementioned nucleic acid molecules. These oligonucleotide probes are preferably of sufficient length to specifically hybridize only to complementary transcripts of the above identified gene(s) of interest under the desired hybridization conditions (e.g., stringent conditions). As used herein, the term "oligonucleotide" refers to a single-stranded nucleic acid. Generally the oligonucleotide probes will be at least 16-20 nucleotides in length, although in some cases longer probes of at least 20-25 nucleotides will be desirable.

[0105] The oligonucleotide probes can be labeled with one or more labeling moieties to permit detection of the hybridized probe/target polynucleotide complexes. Labeling moieties can include compositions that can be detected by spectroscopic, biochemical, photochemical, bioelectronic, immunochemical, electrical optical or chemical means. Examples of labeling moieties include, but are not limited to, radioisotopes, e.g., ³²P, ³³P, ³⁵S, chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectroscopic markers, such as fluorescent markers and dyes, linked enzymes, mass spectrometry tags and magnetic labels.

[0106] Oligonucleotide probe arrays for expression monitoring can be prepared and used according to techniques which are well known to those skilled in the art as described, e.g., in Lockhart et al., *Nature Biotech.*, 14:1675-1680 (1996); McGall et al., *Proc. Natl. Acad. Sci. USA*, 93:13555-13460 (1996); and U.S. Patent No. 6,040,138.

[0107] The invention also provides isolated nucleic acid molecules that are complementary to all the above described isolated nucleic acid molecules.

[0108] An isolated nucleic acid encoding one of the above polypeptides including homologs from species other than mouse or human, may be obtained by a method which comprises the steps of screening an appropriate library under stringent conditions with a labeled probe having the sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 18, or a fragment thereof; and isolating full-length cDNA and genomic clones containing the nucleotide sequences. Such hybridization techniques are well-known to a skilled artisan.

[0109] Nucleic acid molecules of the present invention may be obtained, using standard cloning and screening techniques, from a cDNA library derived from mRNA in cells of the DRG using the expressed sequence tag (EST) analysis (see Adams et al.,

Science, 252:1651-1656 (1991); Adams et al., *Nature*, 355:632-634 (1992); Adams et al., *Nature*, 377:Suppl. 3:174 (1995)). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well-known and commercially available techniques.

[0110] It is also appreciated by one skilled in the art, that an isolated cDNA sequence can be incomplete, in that the region coding for the polypeptide is short at the 5' end of the DNA. This can occur due to the failure of the reverse transcriptase to complete a DNA copy of the mRNA transcript during the synthesis of the first strand of cDNA.

Methods for obtaining full-length cDNAs, or to extend short cDNAs, are well-known in the art, e.g., those based on the method of RACE as described in Frohman et al., *Proc. Natl. Acad. Sci. USA*, 85:8998-9002 (1988). The RACE technique has been modified as

exemplified by Marathon™ technology (Clontech Laboratories, Inc.), wherein cDNAs have been prepared from mRNA extracted from a selected tissues and an adaptor sequence is ligated to each end. Subsequently, nucleic acid amplification (PCR) is carried out to amplify the missing 5'-end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is repeated using primers known as nested

primers that are designed to anneal with the amplified product, which is generally an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence. The reaction products are then analyzed by DNA sequencing and a full-length cDNA is prepared either by directly joining the product to the existing cDNA to provide a complete sequence, or by carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

[0111] When nucleic acid molecules of the present invention are utilized for the recombinant production of polypeptides of the present invention, the polynucleotide can include the coding sequence for the mature polypeptide, by itself, or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, pro- or prepro-protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded, e.g., a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., *Proc. Natl. Acad. Sci. USA*, 86:821-824 (1989), or is an HA tag. The nucleic acid molecule can also contain non-coding 5' and 3'

sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Polypeptides and Antibodies

[0112] In another aspect, the present invention relates to mammalian TRPV3,

TRPV4 and TRPM8 polypeptides. These include the mouse TRPV3 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 2, the human TRPV3 polypeptide comprising an amino acid sequence as set forth in SEQ ID: 5, the mouse TRPV4 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 14, the human TRPV4 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 17, the mouse TRPM8 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO: 8, and the human TRPM8 polypeptide having an amino acid sequence as set forth in SEQ ID NO: 11.

[0113] Further polypeptides of the present invention include isolated polypeptides, i.e., variants, in which the amino acid sequence has at least 90% identity, preferably at least 95% identity, more preferably at least 98% identity and most preferably at least 99% identity, to the amino acid sequences as set forth in SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 14 or SEQ ID NO: 17 over the entire length of these sequences, or a subsequence thereof. Such sequences include the sequences of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 14 and SEQ ID NO: 17.

[0114] The polypeptides of the present invention also include fragments of the aforementioned sequences. For example, the polypeptides of the invention can include amino acids that comprise one or more functional domains of a TRPV3, TRPV4, or TRPM8 polypeptide of the invention. Examples of such domains are described below; other functional domains can be determined using methods known to those of skill in the art.

[0115] The aforementioned TRPV3, TRPV4 and TRPM8 polypeptides can be obtained by a variety of means. Smaller peptides (generally less than 50 amino acids long) may be conveniently synthesized by standard chemical techniques. These polypeptides may also be purified from biological sources by methods well known in the art (see *Protein Purification, Principles and Practice*, 2nd Edition, Scopes, Springer Verlag, NY (1987)). They may also be produced in their naturally occurring, truncated or fusion protein forms by

recombinant DNA technology using techniques well-known in the art. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* genetic recombination (see, e.g., the techniques described in Sambrook et al., *Molecular Cloning, A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Press, NY (2001)); and Ausubel et al., eds., *Short Protocols in Molecular Biology*, 4th Edition, John Wiley & Sons, Inc., NY (1999)). Alternatively, RNA encoding the proteins may be chemically synthesized (see, e.g., the techniques described in *Oligonucleotide Synthesis*, Gait, Ed., IRL Press, Oxford (1984)). Obtaining large quantities of these polypeptides is preferably by recombinant techniques as further described herein.

- 10 [0116] Accordingly, another aspect of the present invention relates to a method for producing a TRPV3, TRPV4 or TRPM8 polypeptide. These methods generally involve:
- a) obtaining a DNA sequence encoding the TRPV3, TRPV4 or TRPM8 polypeptide as defined above; and
 - b) inserting the DNA into a host cell and expressing the TRPV3, TRPV4 or TRPM8 polypeptide. In some embodiments, the methods further include:
 - c) isolating the TRPV3, TRPV4 or TRPM8 polypeptide.

15 [0117] The nucleic acid molecules described herein can be expressed in a suitable host cell to produce active TRPV3, TRPV4 or TRPM8 protein. Expression occurs by placing a nucleotide sequence encoding these proteins into an appropriate expression vector and introducing the expression vector into a suitable host cell, growing the transformed host cell, inducing the expression of one of these proteins, and purifying the recombinant proteins from the host cell to obtain purified, and preferably active, TRPV3, TRPV4 or TRPM8 protein. Appropriate expression vectors are known in the art. For example, pET-14b, pCDNA1 Amp and pVL1392 are available from Novagen and Invitrogen and are suitable vectors for expression in *E. Coli*, COS cells and baculovirus infected insect cells, respectively. These vectors are illustrative of those that are known in the art. Suitable host cells can be any cell capable of growth in a suitable media and allowing purification of the expressed TRPV3, TRPV4 or TRPM8 protein. Examples of suitable host cells include bacterial cells, such as *E. Coli*, *Streptococci*, *Staphylococci*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells, e.g., *Pichia* and *Aspergillus* cells; insect cells, such as *Drosophila S2* and *Spodoptera S9* cells; mammalian cells, such as CHO, COS, HeLa, and plant cells.

[0118] Growth of the transformed host cells can occur under conditions that are known in the art. The conditions will generally depend upon the host cell and the type of vector used. Suitable induction conditions may be used such as temperature and chemicals and will depend on the type of promoter utilized.

- 5 [0119] Purification of the TRPV3, TRPV4 or TRPM8 protein can be accomplished using known techniques without performing undue experimentation. Generally, the transformed cells expressing one of these proteins are broken, crude purification occurs to remove debris and some contaminating proteins, followed by chromatography to further purify the protein to the desired level of purity. Cells can be broken by known techniques such as homogenization, sonication, detergent lysis and freeze-thaw techniques. Crude purification can occur using ammonium sulfate precipitation, centrifugation or other known techniques. Suitable chromatography includes anion exchange, cation exchange, high performance liquid chromatography (HPLC), gel filtration, affinity chromatography, hydrophobic interaction chromatography, etc. Well-known techniques for refolding proteins may be used to obtain the active conformation of the protein when the protein is denatured during intracellular synthesis, isolation or purification.
- 15 [0120] In another aspect, the present invention relates to antibodies that recognize epitopes within the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 14 or SEQ ID NO: 17. As used herein, the term "antibody" includes, but is not limited to, polyclonal antibodies, monoclonal antibodies, humanized or chimeric antibodies and biologically-functional antibody fragments which are those fragments sufficient for binding of the antibody fragment to the protein. Antibodies specific for proteins encoded by the aforementioned sequences have utilities in several types of applications. These may include, e.g., the production of diagnostic kits for use in detecting and diagnosing pain, particularly in differentiating among different types of pain. Another use would be to link such antibodies to therapeutic agents, such as chemotherapeutic agents, followed by administration to subjects suffering from pain. These and other uses are described in more detail below.

20 [0121] For the production of antibodies to a protein encoded by one of the disclosed genes, various host animals may be immunized by injection with the polypeptide, or a portion thereof. Such host animals may include but are not limited to rabbits, mice and rats, to name but a few. Various adjuvants may be used to increase the immunological

response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances, such as lysolecithin, plutonic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants, such as BCG (*Bacille Calmette-Guérin*) and *Corynebacterium parvum*.

[0122] Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals, such as those described above, may be immunized by injection with the encoded protein, or a portion thereof, supplemented with adjuvants as also described above.

[0123] Monoclonal antibodies (mAbs), which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and Milstein, *Nature*, 256:495-497 (1975); and U.S. Patent No. 4,376,110, the human B-cell hybridoma technique (see Kosbor et al., *Immunology Today*, 4:72 (1983); Cole et al., *Proc. Natl. Acad. Sci. USA*, 80:2026-2030 (1983), and the EBV-hybridoma technique (see Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* makes this the presently preferred method of production.

[0124] In addition, techniques developed for the production of "chimeric antibodies" (see Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984); Neuberger et al., *Nature*, 312:604-608 (1984); Takeda et al., *Nature*, 314:452-454 (1985)) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable or hypervariable region derived from a murine mAb and a human immunoglobulin constant region.

[0125] Alternatively, techniques described for the production of single chain antibodies (see U.S. Patent No. 4,946,778; Bird, *Science*, 242:423-426 (1988); Huston et al.,

Proc. Natl. Acad. Sci. USA, 85:5879-5883 (1988); and Ward et al., *Nature*, 334:544-546 (1989)) can be adapted to produce differentially expressed gene single-chain antibodies. Single-chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single-chain polypeptide.

[0126] Most preferably, techniques useful for the production of "humanized antibodies" can be adapted to produce antibodies to the proteins, fragments or derivatives thereof. Such techniques are disclosed in U.S. Patent Nos. 5,932,448; 5,693,762; 5,693,761; 5,583,089; 5,530,101; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,661,016; and 5,770,429.

[0127] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the Fab₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the Fab₂ fragments. Alternatively, Fab expression libraries may be constructed (see Huse et al., *Science*, 246:1275-1281 (1989)) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Assays for Expression of TRPV3, TRPV4 and TRPM8

[0128] In another aspect, diagnostic assays are provided which are capable of detecting the expression of one or more of TRPV3, TRPV4 or TRPM8 in human tissue. Such assays are particularly useful in identifying subjects suffering from pain and differentiating among different types of pain. As stated above, expression of the TRPV3 and TRPV4 genes are up-regulated in a rat injury model. Accordingly, up-regulation of the TRPV3 and TRPV4 genes in a sample obtained from a subject suffering from pain compared with a normal value of expression of these genes, e.g., a sample obtained from a subject not suffering from pain, or a pre-established control for which expression of the gene was determined at an earlier time, is indicative of a subject suffering from pain. Expression of one or more of these genes can be detected by measuring either protein encoded by the gene or mRNA corresponding to the gene in a tissue sample, particularly from a human tissue sample obtained from a site of pain.

[0129] Expression of the TRPV3, TRPV4 and TRPM8 proteins can be detected by a probe which is detectably-labeled, or which can be subsequently-labeled. Generally, the

probe is an antibody which recognizes the expressed protein as described above, especially a monoclonal antibody. Accordingly, in one embodiment, an assay capable of detecting the expression of one or more of TRPV3, TRPV4 or TRPM8 genes comprises contacting a human tissue sample with antibodies preferably monoclonal antibodies, that bind to TRPV3, TRPV4 or TRPM8 polypeptides and determining whether the monoclonal antibodies bind to the polypeptides in the sample.

[0130] Immunoassay methods which utilize the antibodies include, but are not limited to, dot blotting, western blotting, competitive and non-competitive protein binding assays, enzyme-linked immunosorbent assays (ELISA), immunohistochemistry, fluorescence-activated cell sorting (FACS) and others commonly used and widely-described in scientific and patent literature, and many employed commercially.

[0131] Particularly preferred, for ease of detection, is the sandwich ELISA, of which a number of variations exist, all of which are intended to be encompassed by the present invention. For example, in a typical forward assay, unlabeled antibody is

immobilized on a solid substrate and the sample to be tested is brought into contact with the bound molecule, followed by incubation for a period of time sufficient to allow formation of an antibody-antigen binary complex. At this point, a second antibody, labeled with a

reporter molecule capable of inducing a detectable signal, is then added and incubated, allowing time sufficient for the formation of a ternary complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is

determined by observation of a signal, or may be quantitated by comparing with a control sample containing known amounts of antigen. Variations on the forward assay include the simultaneous assay, in which both sample and antibody are added simultaneously to the bound antibody, or a reverse assay in which the labeled antibody and sample to be tested are

first combined, incubated and added to the unlabeled surface bound antibody. These techniques are well-known to those skilled in the art, and the possibility of minor variations will be readily apparent. As used herein, "sandwich assay" is intended to encompass all variations on the basic two-site technique. For the immunoassays of the present invention, the only limiting factor is that the labeled antibody be an antibody which is specific for the protein expressed by the gene of interest, e.g., TRPV3 or a fragment thereof.

[0132] The most commonly used reporter molecules in this type of assay are either enzymes, fluorophore- or radionuclide-containing molecules. In the case of an

enzyme immunoassay an enzyme is conjugated to the second antibody, usually by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different ligation techniques exist, which are well-known to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, among others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine or toluidine are commonly used. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. A solution containing the appropriate substrate is then added to the tertiary complex. The substrate reacts with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an evaluation of the amount of TRPV3, TRPV4 or TRPM8 protein which is present in the tissue sample.

[0133] Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic longer wavelength. The emission appears as a characteristic color visually detectable with a light microscope. Immunofluorescence and EIA techniques are both very well-established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent or bioluminescent molecules may also be employed. It will be readily apparent to the skilled artisan how to vary the procedure to suit the required use.

[0134] The level of expression of mRNA corresponding to the TRPV3, TRPV4 and TRPM8 genes can be detected utilizing methods well-known to those skilled in the art, e.g., northern blotting, RT-PCR, real time quantitative PCR, high density arrays and other hybridization methods. Accordingly, in another embodiment, an assay capable of detecting the expression of one or more of TRPV3, TRPV4 or TRPM8 genes in a sample of tissue, preferably human tissue, is provided which comprises contacting a human tissue sample with an oligonucleotide, i.e., a primer, that is capable of hybridizing to a nucleic acid, particularly

a mRNA, that encodes TRPV3, TRPV4 or TRPM8. The oligonucleotide primer is generally from 10-20 nucleotides in length, but longer sequences can also be employed.

[0135] RNA can be isolated from the tissue sample by methods well-known to those skilled in the art as described, e.g., in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., 1-4.1.1-4.2.9 and 4.5.1-4.5.3 (1996).

[0136] One preferred method for detecting the level of mRNA transcribed from the TRPV3, TRPV4, and TRPM8 genes is RT-PCR. In this method, an mRNA species is first transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase. Methods of reverse transcribing RNA into cDNA are well-known and described in Sambrook et al., *supra*. The cDNA is then amplified as in a standard PCR reaction (referred to as PCR) which is described in detail in U.S. Patent Nos. 4,683,195; 4,683,202; and 4,800,159.

[0137] Briefly, in PCR, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target nucleic acid sequence. An excess of deoxynucleoside triphosphates are added to a reaction mixture along with a DNA polymerase, e.g., Taq polymerase. The primers will bind to the target nucleic acid and the polymerase will cause the primers to be extended along the target nucleic acid sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target nucleic acid to form reaction products, excess primers will bind to the target nucleic acid and to the reaction products and the process is repeated.

[0138] Another preferred method for detecting the level of mRNA transcripts obtained from more than one of the disclosed genes involves hybridization of labeled mRNA to an ordered array of oligonucleotides. Such a method allows the level of transcription of a plurality of these genes to be determined simultaneously to generate gene expression profiles or patterns. In particularly useful embodiments, a gene expression profile derived from a tissue sample obtained from a subject suffering from pain can be compared with a gene expression profile derived from a sample obtained from a normal subject, i.e., a subject not suffering from pain, to determine whether one or more of the TRPV3, TRPV4 and TRPM8 genes are over-expressed in the sample obtained from the subject suffering from pain relative to the genes in the sample obtained from the normal subject, and thereby determine

which gene is responsible for the pain. Ligase chain reaction is another assay that is suitable for detecting the presence of a TRPV3, TRPV4, or TRPM8 polynucleotide.

[0139] The oligonucleotides utilized in this hybridization method typically are bound to a solid support. Examples of solid supports include, but are not limited to, membranes, filters, slides, paper, nylon, wafers, filters, magnetic or nonmagnetic beads, gels, tubing, polymers, polyvinyl chloride dishes, etc. Any solid surface to which the oligonucleotides can be bound, either directly or indirectly, either covalently or non-covalently, can be used. A particularly preferred solid substrate is a high density array or DNA chip. These high density arrays contain a particular oligonucleotide probe in a pre-selected location on the array. Each pre-selected location can contain more than one molecule of the particular probe. Because the oligonucleotides are at specified locations on the substrate, the hybridization patterns and intensities (which together result in a unique expression profile or pattern) can be interpreted in terms of expression levels of particular genes.

[0140] The oligonucleotide probes are preferably of sufficient length to specifically hybridize only to complementary transcripts of the above identified gene(s) of interest. As used herein, the term "oligonucleotide" refers to a single-stranded nucleic acid. Generally the oligonucleotides probes will be at least 16-20 nucleotides in length, although in some cases longer probes of at least 20-25 nucleotides will be desirable.

[0141] The oligonucleotide probes can be labeled with one or more labeling moieties to permit detection of the hybridized probe/target polynucleotide complexes. Labeling moieties can include compositions that can be detected by spectroscopic, biochemical, photochemical, bioelectronic, immunochemical, electrical optical or chemical means. Examples of labeling moieties include, but are not limited to, radioisotopes, e.g., ^{32}P , ^{33}P , ^{35}S , chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectroscopic markers, such as fluorescent markers and dyes, linked enzymes, mass spectrometry tags and magnetic labels.

[0142] Oligonucleotide probe arrays for expression monitoring can be prepared and used according to techniques which are well-known to those skilled in the art as described, e.g., in Lockhart et al., *supra*; McGall et al., *supra*; and U.S. Patent No. 6,040,138.

[0143] In another aspect, kits are provided for detecting the level of expression of one or more of the TRPV3, TRPV4 and TRPM8 genes in a sample of tissue, e.g., a sample of tissue from a site of pain. For example, the kit can comprise a labeled compound or agent capable of detecting a protein encoded by, or mRNA corresponding to, at least one of the genes TRPV3, TRPV4 and TRPM8; or fragment of the protein, means for determining the amount of protein encoded by or mRNA corresponding to the gene or fragment of the protein; and means for comparing the amount of protein encoded by or mRNA corresponding to the gene or fragment of the protein, obtained from the subject sample with a standard level of expression of the gene, e.g., from a sample obtained from a subject not suffering pain. With respect to detection of TRPV3, TRPV4 and TRPM8 proteins, the agent can be an antibody specific for these proteins. With respect to detection of mRNA, the agent can be pre-selected primer pairs that selectively hybridize to mRNA corresponding to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 18. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect protein encoded by or mRNA corresponding to the gene.

[0144] In another aspect, the present invention is based on the identification of novel genes that are specific for trkA^+ pain-specific DRG neurons. DRG neurons can be classified into several distinct subpopulations with different functional, biochemical and morphological characteristics. The only known early markers differentially expressed by the DRG subtypes are the *trk* family of neurotrophin receptors. Gene-targeted deletion of the mouse neurotrophins and *trks* (receptor tyrosine kinases) have demonstrated that neurotrophin signaling is required for the survival of the different subpopulations of DRG neurons that *trks* specifically mark. For example, *trkA* knockout mice lack the nociceptive and thermosensitive neurons that sense pain and temperature.

Identification of Agonists and Antagonists

[0145] In another aspect, the present invention relates to the use of the TRPV3, TRPV4 and TRPM8 genes in methods for identifying agents useful in treating pain, or modulating responses to heat and cold, as flavor enhancers (e.g., menthol mimetics that one can identify using TRPM8 in a screening assay) and as cosmetic additives that provide a

cool or warm sensation to the skin (e.g., menthol mimetics, capsaicin mimetics or other compounds identified using TRPM8 or TRPV3 in screening assays). These methods comprise assaying for the ability of various agents to bind and/or modulate the activity of the proteins encoded by these genes, and/or decrease or increase the level of expression of mRNA corresponding to or protein encoded by these genes. The candidate agent may function as an antagonist or agonist. Examples of various candidate agents include, but are not limited to, natural or synthetic molecules such as antibodies, proteins or fragments thereof, antisense nucleotides, double-stranded RNA, ribozymes, organic or inorganic compounds, etc. Methods for identifying such candidate agents can be carried out in cell-based systems and in animal models.

[0146] For example, proteins encoding these genes expressed in a recombinant host cell such as CHO or COS may be used to identify candidate agents that bind to and/or modulate the activity of the protein, or that increase or decrease the level of expression of mRNA corresponding to or encoded by these genes. In this regard, the specificity of the binding of a candidate agent showing affinity for the protein can be shown by measuring the affinity of the agents for cells expressing the receptor or membranes from these cells. This can be achieved by measuring the specific binding of labeled, e.g., radioactive agent to the cell, cell membranes or isolated protein, or by measuring the ability of the candidate agent to displace the specific binding of standard labeled ligand.

[0147] Cells expressing proteins encoded by these genes can also be utilized to identify agents that modulate the protein's activity. For example, one method for identifying compounds useful for treating pain, or for use as a flavor or fragrance, comprises, providing a cell that expresses one of these proteins, e.g., TRPV3, TRPV4 or TRPM8, combining a candidate agent with the cell and measuring the effect of the candidate agent on the protein's activity. The cell can be a mammalian cell, a yeast cell, bacterial cell, insect cell or any other cell expressing the TRPV3 protein. The candidate compound is evaluated for its ability to elicit an appropriate response, e.g., the stimulation of cellular depolarization or increase in intracellular calcium ion levels due to calcium ion influx.

[0148] The level of intracellular calcium can be assessed using a calcium ion-sensitive fluorescent indicator such as a calcium ion-sensitive fluorescent dye, including, but not limited to, quin-2 (see, e.g., Tsien et al., *J. Cell Biol.*, 94:325 (1982)), fura-2 (see, e.g., Grynkiewicz et al., *J. Biol. Chem.*, 260:3440 (1985)), fluo-3 (see, e.g., Kao et al., *J. Biol.*

Chem., 264:8179 (1989)) and rhod-2 (see, e.g., Tsien et al., *J. Biol. Chem.*, Abstract 89a (1987)).

[0149] Membrane depolarization of recombinant cells expressing the above proteins can be monitored using a fluorescent dye that is sensitive to changes in membrane potential, including, but not limited to, carbocyanines such as 3,3'-dipentylloxacarbocyanine iodide (DiO₆) and 3,3'-dipropylthiadicarbocyanine iodide (DiSC₃), oxonols, such as bis-(1,3-dibutylbarbituric acid) pentamethine oxonol (DiBAC₄ (Biotrend Chemikalien GmbH, Cologne, Germany)) or bis-(1,3-dibutylbarbituric acid) pentamethine oxonol, etc. Cellular fluorescence can be monitored using a fluorometer.

10 [0150] The assays to identify antagonists of ion channel activity are preferably performed under conditions in which the particular ion channel is active. Conversely, when seeking to identify an agonist, one would preferably perform the screening under conditions in which the ion channel is not active in the absence of the agonist. For example, TRPV3 is activated (i.e., mediates ion passage through a membrane) at temperatures of about 33°C and above. Accordingly, it is preferred to screen for antagonists of TRPV3 at a temperature of above about 33°C (e.g., 35°, 40°, 45°, or above), and to screen for agonists of TRPV3 at a temperature below 33°C (e.g., 30°, 25°, 20°C, or below). In some assays, it is desirable to discriminate between TRPV3-mediated ion transport and ion transport mediated by a different TRP ion channel. For example, to discriminate between TRPV3-mediated cation transport and cation transport mediated by, for example, TRPV1 or TRPV2, the assay can be conducted at a temperature above the activation threshold of TRPV3 but below the activation threshold of the other receptor (e.g., below about 43°C or below about 52°C, respectively, for TRPV1 and TRPV2). Thus, an assay temperature of between about 35°C and about 40°C would result in active TRPV3, but inactive TRPV1 and TRPV2.

25 [0151] Similarly, assays to identify antagonists of TRPM8 cation channel activity are preferably conducted under conditions in which the TRPM8 conducts cations in the absence of an antagonist. For example, since the threshold activation temperature of TRPM8 is approximately 15°C, one could screen for antagonists at a temperature below 15°C (e.g., 10°, 5°, 0°C, and the like). TRPM8 also is activated by menthol, so instead of or in addition to regulating activity by temperature, one could conduct the assay for antagonists in the presence of menthol. To identify an agonist of TRPM8, it is preferred to conduct the assay under conditions in which TRPM8 does not exhibit significant ion channel activity, such as a

temperature above 15°C (e.g., 20°C, 25°C, 30°C, etc.). To distinguish between TRPM8-mediated cation channel activity and that of other TRP ion channels, the assay for agonists can be conducted at a temperature below 33°C (the activation temperature of TRPV3). For example, a temperature between 20°C and 30°C would result in TRPV3 being inactive in the absence of an agonist, and TRPV3, TRPV1 and TRPV2 also being inactive.

5 [0152] The TRPV3, TRPV4, and TRPV8 cation channels function to transport not only divalent cations (e.g., Ca²⁺), but also monovalent cations (e.g., Na⁺, K⁺).

10 [0153] The assay can be carried out manually or using an automated system. For high throughput screening assays to identify ligands of such proteins, an automated system is preferred. For example, one type of automated system provides a 96-well, 384-well, or 1536-well, culture plate wherein a recombinant cell comprising a nucleotide sequence encoding such a protein is cultured to express the protein. The plate is loaded into a fluorescence imaging plate reader (e.g., "FLIPR" commercially available from Molecular Devices Corp., Sunnyvale, CA) which measure the kinetics of intracellular calcium influx in each of the wells. The FLIPR[®] can quantitatively transfer fluids into and from each well of the plate and thus can be utilized to add the calcium-ion sensitive fluorescent indicator dye, a candidate agent, etc. Membrane potential dyes suitable for high throughput assays include the FLIPR[®] Membrane Potential Assay Kit as sold by Molecular Devices Corp.

15 [0154] Once a candidate compound is identified as an agonist, such agonists can be added to cells expressing such proteins followed by the addition of various candidate agents to determine which agents function as antagonists.

20 [0155] The nucleic acids and polypeptides of the present invention can also be utilized to identify candidate agents that modulate, i.e., increase or decrease the level of expression of mRNA and proteins in cells expressing these proteins. For example, expression of the TRPV4 gene is shown to be up-regulated in a rat injury model (see Example 3). The level of expression of mRNA and protein can be detected utilizing methods well-known to those skilled in the art as described above.

25 [0156] After initial screening assays have identified agents that inhibit the protein's activity or level of expression of mRNA or protein, these agents can then be assayed in conventional live animal models of pain to assess the ability of the agent to ameliorate the pathological effects produced in these models and/or inhibit expression levels of mRNA or protein. For example, in the case of the TRPV4 gene which is shown to be up-

regulated in a rat injury model, one method for identifying an agent useful in the treatment of pain comprises:

- a) administering a candidate agent, e.g., an antisense nucleotide derived from the sequence of the TRPV4 gene, to a subject such as a rat model of pain; and
- b) determining reversal of established pain in the animal. Various animal models utilized in neuropathic pain are well-known in the art, e.g., the partial sciatic ligation model, i.e., the Seltzer model, the chronic constriction injury model, i.e., the CCI model and the spinal nerve ligation model, i.e., the Chung model.

[0157] For example, in the partial sciatic ligation (see, the Seltzer model as described in Seltzer et al., *Pain*, 43:205-218 (1990)), rats are anesthetized and a small incision made mid-way up one thigh (usually the left) to expose the sciatic nerve. The nerve is carefully cleared of surrounding connective tissues at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. A 7-0 silk suture is inserted into the nerve with a 3/8 curved, reversed-cutting mini-needle, and tightly ligated so that the dorsal 1/3 to 1/2 of the nerve thickness is held within the ligature. The muscle and skin are closed with sutures and clips and the wound dusted with antibiotic powder. In sham animals the sciatic nerve is exposed but not ligated and the wound closed as before.

[0158] In the chronic constriction model (the CCI model as described in Bennett et al., *Pain*, 33:87-107 (1988)) rats are anesthetized and a small incision is made midway up one thigh to expose the sciatic nerve. The nerve is freed of surrounding connective tissue and four ligatures of chromic gut are tied loosely around the nerve with approximately 1 mM between each, so that the ligatures just barely construct the surface of the nerve. The wound is closed with sutures and clips. In sham animals the sciatic nerve is exposed but not ligated and the wound is closed.

[0159] In the spinal nerve ligation (see, the Chung model as described in Kim et al., *Pain*, 50:355-363 (1992)) rats are anesthetized and placed into a prone position and an incision made to the left of the spine at the L4-S2 level. A deep dissection through the paraspinal muscles and separation of the muscles from the spinal processes at the L4-S2 level will reveal part of the sciatic nerve as it branches to form the L4, L5 and L6 spinal nerves. The L6 transverse process is carefully removed with a small rongeur enabling visualization of these spinal nerves. The L5 spinal nerve is isolated and tightly ligated with

7-0 silk suture. The wound is closed with a single muscle suture (6-0 silk) and one or two skin closure clips and dusted with antibiotic powder. In sham animals the L5 nerve is exposed as before but not ligated and the wound closed as before.

[0160] Male Wistar rats (120-140 g) are used for each of the three models.

- 5 Mechanical hyperalgesia is then assessed in rat by measuring paw withdrawal thresholds of both hindpaws to an increasing pressure stimulus using an Analgesymeter (Ugo-Basile, Milan). Thermal hyperalgesia is assessed by measuring withdrawal latencies to a noxious thermal stimuli applied to the underside of each hindpaw. With all models, mechanical hyperalgesia and allodynia and thermal hyperalgesia develop within 1-3 days following surgery and persist for at least 50 days. Reversal of mechanical hyperalgesia and allodynia and thermal hyperalgesia is assessed following administration of the agent, e.g., the antisense nucleotide specific for the TRPV4 gene.

[0161] Another example of a method for identifying agents useful in treating pain comprises:

- 15 a) administering a candidate agent to a subject such as a rat model of pain;
- b) detecting a level of expression of a protein encoded by or mRNA corresponding to one of genes described herein, e.g., TRPV4, in a sample obtained from the subject; and
- c) comparing the level of expression of the protein or mRNA in the sample in the presence of the agent with a level of expression of the protein or mRNA obtained from the sample of the subject in the absence of the agent, wherein a decreased level of expression of the protein or mRNA in the sample in the presence of the agent relative to the level of expression of the protein or mRNA in the absence of the agent is indicative that the agent is useful in the treatment of pain.

[0162] The present invention also provides a method for identifying an agent useful in the modulation of a mammalian sensory response. The method comprises

- 25 a) contacting a candidate agent with a test system that comprises a receptor polypeptide selected from the group consisting of TRPM8, TRPV3, and TRPV4; and
- b) detecting a change in activity of the receptor polypeptide in the presence of the candidate agent as compared to the activity of the receptor polypeptide in the absence of the agent, thereby identifying an agent that modulates receptor activity.

[0163] In particularly useful embodiments of this method, the sensory response is response to cold and the polypeptide is a TRPM8 polypeptide preferably having an amino

acid sequence selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 11.

The method can further include the step of administering the agent that modulates receptor activity to a test subject, and thereafter detecting a change in the sensory response in the test subject.

- 5 [0164] The test system that is contacted with a candidate agent can comprise, e.g., a membrane that comprises the receptor polypeptide or a cell that expresses a heterologous polynucleotide that encodes the receptor polypeptide. In a useful embodiment, the heterologous polynucleotide comprises a nucleotide sequence as set forth in nucleotides 448-3762 of SEQ ID NO: 7 or as set forth in nucleotides 61-4821 of SEQ ID NO: 10, and the receptor polypeptide is a TRPM8 polypeptide. The cell can be substantially isolated wherein the step of contacting of the cell with the candidate agent is performed *in vitro* or the cell can be present in an organism wherein the step of contacting is performed *in vivo*.

- 10 [0165] In particularly useful embodiments, the receptor activity comprises increased or decreased Ca^{2+} passage through the membrane comprising the receptor polypeptide, wherein the membrane can be, e.g., a substantially purified cell membrane or a membrane comprising a liposome.

Pharmaceutical Compositions and Methods

- 15 [0166] The present invention also provides for therapeutic methods of treating a subject suffering from pain utilizing the aforementioned genes, i.e., TRPV3, TRPV4, and TRPM8. Examples of suitable therapeutic agents include, but are not limited to, antisense nucleotides, ribozymes, double-stranded RNAs, antagonists and agonists, as described in detail below.

- 20 [0167] As used herein, the term "antisense" refers to nucleotide sequences that are complementary to a portion of an RNA expression product of at least one of the disclosed genes. "Complementary" nucleotide sequences refer to nucleotide sequences that are capable of base-pairing according to the standard Watson-Crick complementary rules. That is, purines will base pair with pyrimidine to form combinations of guanine:cytosine and adenine:thymine in the case of DNA, or adenine:uracil in the case of RNA. Other less common bases, e.g., inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others may be included in the hybridizing sequences and will not interfere with pairing.

[0168] When introduced into a host cell, antisense nucleotide sequences specifically hybridize with the cellular mRNA and/or genomic DNA corresponding to the gene(s) so as to inhibit expression of the encoded protein, e.g., by inhibiting transcription and/or translation within the cell.

- 5 [0169] The isolated nucleic acid molecule comprising the antisense nucleotide sequence can be delivered, e.g., as an expression vector, which when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the encoded mRNA of the gene(s). Alternatively, the isolated nucleic acid molecule comprising the antisense nucleotide sequence is an oligonucleotide probe which is prepared *ex vivo* and, which when introduced into the cell results in inhibiting expression of the encoded protein by hybridizing with the mRNA and/or genomic sequences of the gene(s).

- 10 [0170] Preferably, the oligonucleotide contains artificial internucleotide linkages which render the antisense molecule resistant to exonucleases and endonucleases, and thus are stable in the cell. Examples of modified nucleic acid molecules for use as antisense nucleotide sequences are phosphoramidate, phosphorothioate and methylphosphonate analogs of DNA as described, e.g., in U.S. Patent Nos. 5,176,996; 5,264,564, and 5,256,775.

15 General approaches to preparing oligomers useful in antisense therapy are described, e.g., in Van der Krol, *BioTechniques*, 6:958-976 (1988), and Stein et al., *Cancer Res.*, 48:2659-2668 (1988).

- 20 [0171] Typical antisense approaches, involve the preparation of oligonucleotides, either DNA or RNA, that are complementary to the encoded mRNA of the gene. The antisense oligonucleotides will hybridize to the encoded mRNA of the gene and prevent translation. The capacity of the antisense nucleotide sequence to hybridize with the desired gene will depend on the degree of complementarity and the length of the antisense nucleotide sequence. Typically, as the length of the hybridizing nucleic acid increases, the more base mismatches with an RNA it may contain and still form a stable duplex or triplex. One skilled in the art can determine a tolerable degree of mismatch by use of conventional procedures to determine the melting point of the hybridized complexes.

- 25 [0172] Antisense oligonucleotides are preferably designed to be complementary to the 5' end of the mRNA, e.g., the 5' untranslated sequence up to and including the regions complementary to the mRNA initiation site, i.e., AUG. However, oligonucleotide sequences that are complementary to the 3' untranslated sequence of mRNA have also been shown to

be effective at inhibiting translation of mRNAs as described e.g. in Wagner, *Nature*, 372:333 (1994). While antisense oligonucleotides can be designed to be complementary to the mRNA coding regions, such oligonucleotides are less efficient inhibitors of translation.

[0173] Regardless of the mRNA region to which they hybridize, antisense

oligonucleotides are generally from about 15 to about 25 nucleotides in length.

[0174] The antisense nucleotide can also comprise at least one modified base moiety, e.g., 3-methylcytosine, 5-methylcytosine, 7-methylguanine, 5-fluorouracil,

5-bromouracil and may also comprise at least one modified sugar moiety, e.g., arabinose, hexose, 2-fluorarabinose and xylose.

[0175] In another embodiment, the antisense nucleotide sequence is an

alpha-anomeric nucleotide sequence. An alpha-anomeric nucleotide sequence forms specific double stranded hybrids with complementary RNA, in which, contrary to the usual beta-units, the strands run parallel to each other as described e.g. in Gautier et al., *Nucl. Acids. Res.*, 15:6625-6641 (1987).

[0176] Antisense nucleotides can be delivered to cells which express the described genes *in vivo* by various techniques, e.g., injection directly into the target tissue site, entrapping the antisense nucleotide in a liposome, by administering modified antisense nucleotides which are targeted to the target cells by linking the antisense nucleotides to peptides or antibodies that specifically bind receptors or antigens expressed on the cell surface.

[0177] However, with the above-mentioned delivery methods, it may be difficult to attain intracellular concentrations sufficient to inhibit translation of endogenous mRNA.

Accordingly, in a preferred embodiment, the nucleic acid comprising an antisense nucleotide sequence is placed under the transcriptional control of a promoter, i.e., a DNA sequence which is required to initiate transcription of the specific genes, to form an expression construct. The use of such a construct to transfect cells results in the transcription of

sufficient amounts of single-stranded RNAs to hybridize with the endogenous mRNAs of the described genes, thereby inhibiting translation of the encoded mRNA of the gene. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of the antisense nucleotide sequence. Such vectors can be constructed by standard recombinant technology methods. Typical expression vectors include bacterial plasmids or phage, such as those of the pUC or Bluescript[™] plasmid series, or viral vectors

such as adenovirus, adeno-associated virus, herpes virus, vaccinia virus and retrovirus, adapted for use in eukaryotic cells. Expression of the antisense nucleotide sequence can be achieved by any promoter known in the art to act in mammalian cells. Examples of such

promoters include, but are not limited to, the promoter contained in the 3' long terminal

5 repeat of Rous sarcoma virus as described, e.g., in Yamamoto et al., *Cell*, 22:787-797

(1980); the herpes thymidine kinase promoter as described, e.g., in Wagner et al., *Proc. Natl. Acad. Sci. USA*, 78:1441-1445 (1981); the SV40 early promoter region as described e.g., in Bernoist and Chambon, *Nature*, 290:304-310 (1981); and the regulatory sequences of the metallothionein gene as described, e.g., in Brinster et al., *Nature*, 296:39-42 (1982).

[0178] Ribozymes are RNA molecules that specifically cleave other single-stranded RNA in a manner similar to DNA restriction endonucleases. By modifying the nucleotide sequences encoding the RNAs, ribozymes can be synthesized to recognize specific nucleotide sequences in a molecule and cleave it as described, e.g., in Cech, *J. Amer. Med. Assn.*, 260:3030 (1988). Accordingly, only mRNAs with specific sequences are

15 cleaved and inactivated.

[0179] Two basic types of ribozymes include the "hammerhead" type as described, e.g., in Rossie et al., *Pharmac. Ther.*, 50:245-254 (1991); and the hairpin ribozyme as described, e.g., in Hampel et al., *Nucl. Acids Res.*, 18:299-304 (1990) and U.S. Patent No. 5,254,678. Intracellular expression of hammerhead and hairpin ribozymes

20 targeted to mRNA corresponding to at least one of the disclosed genes can be utilized to inhibit protein encoded by the gene.

[0180] Ribozymes can either be delivered directly to cells, in the form of RNA oligonucleotides incorporating ribozyme sequences, or introduced into the cell as an expression vector encoding the desired ribozymal RNA. Ribozyme sequences can be modified in essentially the same manner as described for antisense nucleotides, e.g., the

25 ribozyme sequence can comprise a modified base moiety.

[0181] Double-stranded RNA, i.e., sense-antisense RNA, corresponding to at least one of the disclosed genes can also be utilized to interfere with expression of at least one of the disclosed genes. Interference with the function and expression of endogenous genes by double-stranded RNA has been shown in various organisms such as *C. elegans* as described e.g., in Fire et al., *Nature*, 391:806-811 (1998); *Drosophila* as described, e.g., in Kennerdell et al., *Cell*, 23:95(7):1017-1026 (1998); and mouse embryos as described, e.g., in Wianni et

al., *Nat. Cell Biol.*, 2(2):70-75 (2000). Such double-stranded RNA can be synthesized by *in vitro* transcription of single-stranded RNA read from both directions of a template and *in vitro* annealing of sense and antisense RNA strands. Double-stranded RNA can also be synthesized from a cDNA vector construct in which the gene of interest is cloned in opposing orientations separated by an inverted repeat. Following cell transfection, the RNA is transcribed and the complementary strands reanneal. Double-stranded RNA corresponding to at least one of the disclosed genes could be introduced into a cell by cell transfection of a construct such as that described above.

[0182] The term "antagonist" with respect to methods of treatment refers to a molecule which, when bound to the protein encoded by the gene, inhibits its activity.

Antagonists can include, but are not limited to, peptides, proteins, carbohydrates and small molecules (generally, a molecule having a molecular weight of about 1000 daltons or less).

[0183] The term "agonist" with respect to methods of treatment refers to a molecule which, when bound to the protein encoded by the gene, activates its activity.

Agonists can include, but are not limited to, peptides, proteins, carbohydrates and small molecules.

[0184] In a particularly useful embodiment, the antagonist is an antibody-specific for the cell-surface protein expressed by one of the genes, e.g., TRPV3. Antibodies useful as therapeutics encompass the antibodies as described above, and are preferably monoclonal antibodies. The antibody alone may act as an effector of therapy or it may recruit other cells to actually effect cell killing. The antibody may also be conjugated to a reagent such as a chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc. and serve as a target agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor target. Various effector cells include, cytotoxic T cells and NK cells.

[0185] Examples of the antibody-therapeutic agent conjugates which can be used in therapy include, but are not limited to: 1) antibodies coupled to radionuclides, such as ^{125}I , ^{131}I , ^{123}I , ^{111}In , ^{153}Sm , ^{67}Cu , ^{64}Ga , ^{186}Ho , ^{177}Lu , ^{188}Re and ^{188}Re , and as described, e.g., in Goldenberg et al., *Cancer Res.*, 41:4354-4360 (1981); Carrasquillo et al., *Cancer Treat. Rep.*, 68:317-328 (1984); Zalberg et al., *J. Natl. Cancer Inst.*, 72:697-704 (1984); Jones et al., *Int. J. Cancer*, 35:715-720 (1985); Lange et al., *Surgery*, 98:143-150 (1985); Kallovich et al., *J. Nucl. Med.*, 27:897 (1986); Order et al., *Int. J. Radiother. Oncol. Biol.*

Phys., 8:259-261 (1982); Courtemay-Luck et al., *Lancet*, 1:1441-1443 (1984) and Ellinger et al., *Cancer Treat. Rep.*, 66:289-297 (1982); 2) antibodies coupled to drugs or biological response modifiers, such as methotrexate, adriamycin and lymphokines, such as interferon as described, e.g., in Chabner et al., *Cancer, Principles and Practice of Oncology*,

J.B. Lippincott Co., Philadelphia, PA, 1:290-328 (1983); Oldham et al., *Cancer, Principles and Practice of Oncology*, J.B. Lippincott Co., Philadelphia, PA, 2:2223-2245 (1985);

Deguchi et al., *Cancer Res.*, 46:3751-3755 (1986); Deguchi et al., *Fed. Proc.*, 44:1684 (1985); Embleton et al., *Br. J. Cancer*, 49:559-565 (1984); and Pimm et al., *Cancer Immunol. Immunother.*, 12:125-134 (1982); 3) antibodies coupled to toxins, as described,

e.g., in Uhr et al., *Monoclonal Antibodies and Cancer*, Academic Press, Inc., pp. 85-98

(1983); Vilella et al., *Biotechnology and Bio. Frontiers*, P.H. Abelson, Ed., pp. 73-85 (1984) and Vilella et al., *Science*, 219:644-650 (1983); 4) heterofunctional antibodies, for example,

antibodies coupled or combined with another antibody so that the complex binds both to the carcinoma and effector cells, e.g., killer cells, such as T cells, as described, e.g., in Perez et al., *J. Exper. Med.*, 163:166-178 (1986); and Lau et al., *Proc. Natl. Acad. Sci. USA*,

82:8648-8652 (1985); and 5) native, i.e., non-conjugated or non-complexed, antibodies, as described in, e.g., in Hertlyn et al., *Proc. Natl. Acad. Sci. USA*, 79:4761-4765 (1982); Schultz et al., *Proc. Natl. Acad. Sci. USA*, 80:5407-5411 (1983); Capoue et al., *Proc. Natl. Acad. Sci. USA*, 80:7328-7332 (1983); Sears et al., *Cancer Res.*, 45:5910-5913 (1985); Nepom et al.,

Proc. Natl. Acad. Sci. USA, 81:2864-2867 (1984); Koprowski et al., *Proc. Natl. Acad. Sci. USA*, 81:216-219 (1984); and Houghton et al., *Proc. Natl. Acad. Sci. USA*, 82:1242-1246

(1985).

[0186] Methods for coupling an antibody or fragment thereof to a therapeutic agent as described above are well-known in the art and are described, e.g., in the methods provided in the references above. In yet another embodiment, the antagonist useful as a therapeutic for treating disorders can be an inhibitor of a protein encoded by one of the disclosed genes.

[0187] In the case of treatment with an antisense nucleotide, the method comprises administering a therapeutically effective amount of an isolated nucleic acid molecule comprising an antisense nucleotide sequence derived from at least one of the disclosed genes, wherein the antisense nucleotide has the ability to decrease the transcription/translation of one of the genes. The term "isolated" nucleic acid molecule

means that the nucleic acid molecule is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring nucleic acid molecule is not isolated, but the same nucleic acid molecule, separated from some or all of the coexisting materials in the natural system, is isolated, even if subsequently reintroduced into the natural system. Such nucleic acid molecules could be part of a vector or part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

[0188] With respect to treatment with a ribozyme or double-stranded RNA molecule, the method comprises administering a therapeutically effective amount of a nucleotide sequence encoding a ribozyme, or a double-stranded RNA molecule, wherein the nucleotide sequence encoding the ribozyme/double-stranded RNA molecule has the ability to decrease the transcription/translation of one of the genes.

[0189] In the case of treatment with an antagonist, the method comprises administering to a subject a therapeutically effective amount of an antagonist that inhibits a protein encoded by one of these genes.

[0190] In the case of treatment with an agonist, the method comprises administering to a subject a therapeutically effective amount of an agonist that inhibits a protein encoded by one of these genes. In particularly useful embodiments, the gene is TRPV8, and the agonist can include compounds that are derivatives of menthol and other compounds known to be cool-feeling agents including, but not limited to, camphor, thymol, peppermint oil, thymol and the like. Such compounds can be particularly useful in alleviating pain associated with skin inflammation by providing a cool sensation to the skin.

[0191] A "therapeutically effective amount" of an isolated nucleic acid molecule comprising an antisense nucleotide, nucleotide sequence encoding a ribozyme, double-stranded RNA, agonist or antagonist, refers to a sufficient amount of one of these therapeutic agents to treat a subject suffering from pain. The determination of a therapeutically effective amount is well within the capability of those skilled in the art. For any therapeutic, the therapeutically effective dose can be estimated initially either in cell culture assays, or in animal models, usually mice, rats, rabbits, dogs or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

[0192] The present invention also provides for methods of treating pain, wherein the method comprises identifying a patient suffering from a TRPV3-, TRPV4- or TRPM8-mediated pain by measuring expression of protein encoded by or mRNA corresponding to the TRPV3, TRPV4 or TRPM8 gene, and then administering to such a patient an analgesically effective amount of an agent which decreases or increases the activity or expression of one of these genes. The agent can be a therapeutic agent as described above.

[0193] An "analgesically effective amount" can be a therapeutically effective amount as described above.

[0194] Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀. Antisense nucleotides, ribozymes, double-stranded RNAs, agonists, antagonists and other agents which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient and the route of administration.

[0195] The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy.

[0196] Normal dosage amounts may vary from 0.1-100,000 mg. up to a total dose of about 1 g. depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for antagonists.

[0197] For therapeutic applications, the antisense nucleotides, nucleotide sequences encoding ribozymes, double-stranded RNAs (whether entrapped in a liposome or

contained in a viral vector), antibodies or other agents are preferably administered as pharmaceutical compositions containing the therapeutic agent in combination with one or more pharmaceutically acceptable carriers. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

[0198] The pharmaceutical compositions may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intraarticular, intraarterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal means.

[0199] In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences, Maack Publishing Co., Easton, PA.

[0200] Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well-known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for ingestion by the patient.

[0201] Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol or sorbitol; starch from corn, wheat, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof, such as sodium alginate.

[0202] Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyes/stuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

[0203] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid or liquid polyethylene glycol with or without stabilizers.

[0204] Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers, such as Hank's solution, Ringer's solution or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil or synthetic fatty acid esters, such as ethyl oleate or triglycerides or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0205] For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0206] The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, enterprising or lyophilizing processes.

[0207] The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1-2% sucrose, and 2-7% mannitol, at a pH range of 4.5-5.5, that is combined with buffer prior to use.

[0208] After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of the antisense nucleotide or antagonist, such labeling would include amount, frequency and method of administration. Those skilled in the art will employ different formulations for antisense nucleotides than for antagonists, e.g., antibodies or inhibitors. Pharmaceutical formulations suitable for oral administration of proteins are described, e.g., in U.S. Patent Nos. 5,008,114; 5,505,962; 5,641,515; 5,681,811; 5,700,486; 5,766,633; 5,792,451; 5,853,748; 5,972,387; 5,976,569; and 6,051,561.

[0209] In another aspect, the treatment of a subject, e.g., a rat injury model, with a therapeutic agent such as those described above, can be monitored by detecting the level of expression of mRNA or protein encoded by at least one of the disclosed genes, or the activity of the protein encoded by the gene. These measurements will indicate whether the treatment is effective or whether it should be adjusted or optimized. Accordingly, one or more of the genes described herein can be used as a marker for the efficacy of a drug during clinical trials.

[0210] In a particularly useful embodiment, a method for monitoring the efficacy of a treatment of a subject suffering from pain with an agent (e.g., an antagonist, protein, nucleic acid, small molecule or other therapeutic agent or candidate agent identified by the screening assays described herein) is provided comprising:

- a) obtaining a pre-administration sample from a subject prior to administration of the agent;
- b) detecting the level of expression of mRNA or protein encoded by the gene, or activity of the protein encoded by the gene in the pre-administration sample;
- c) obtaining one or more post-administration samples from the subject;

- d) detecting the level of expression of mRNA or protein encoded by the gene, or activity of the protein encoded by the gene in the post-administration sample or samples;
- e) comparing the level of expression of expression of mRNA or protein encoded by the gene, or activity of the protein encoded by the gene in the pre-administration sample with the level of expression of mRNA or protein encoded by the gene, or activity of the protein encoded by the gene in the post-administration sample or samples; and
- f) adjusting the administration of the agent accordingly.

[0211] For example, increased administration of the agent may be desirable to decrease the level of expression or activity of the gene to lower levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to increase expression or activity of the gene to higher levels than detected, i.e., to decrease the effectiveness of the agent.

EXAMPLES

[0212] The following examples are offered to illustrate, but not to limit the present invention.

EXAMPLE 1

Identification of New VRs

A. VR searching

[0213] Strategy: Known VR sequences are downloaded (GI Nos. 6782444, 5305598, 7106445, 4589143, 6635238, 2570933, 5263196 and 4589141) from NCBI and assembled using Clustal (Megalign--DNAslar, Madison, WI) with the following parameters: Gap Penalty 10, GapLength Penalty 10, Ktuple 1, Window 5 and Diagonals Saved 5. This alignment is saved as a *.MSF file.

[0214] This *.MSF file is converted to a hidden Markov model using HMMBUILD 2.0 (Sean Eddy, Washington University, St. Louis) then calibrated using HMMCALIBRATE 2.0 (Sean Eddy), and used to search 6 frame translations (Feb 20 release) of the Celera human genome data using the default parameters. The protein sequences of these files are retrieved and used as subjects in a BLASTP search of NR. This file is manually inspected identifying three novel candidates for VRs.

B. Identification of VR TRPV3

[0215] Mechanical and thermal stimuli activate specialized sensory neurons that terminate in the skin at receptor structures like hair follicles or as free nerve endings. Pain and temperature sensitive neurons belong to the latter category and are thus thought to directly sense stimuli. A TRP channel that is expressed in pain neurons, VR1 is partially responsible for the detection of noxious heat. This Example describes the cloning of TRPV3, a close relative of VR1 that is also activated by noxious heat. Surprisingly, TRPV3 is most highly-expressed in skin cells. Keratinocytes that express TRPV3 show a calcium influx in response to noxious heat. Therefore, skin cells possess molecular tools similar to those of sensory neurons to "sense" heat.

[0216] VR1 (TRPV1), the best-characterized receptor in the somatic sensory system, is directly gated by noxious heat. VR1 is expressed in small-diameter, nociceptive DRG neurons that terminate in the skin as free nerve endings to detect noxious heat.

Analysis of VR1 knockout mice has demonstrated that this channel is partially responsible for heat sensitivity. VR1 belongs to the family of six transmembrane-containing TRP non-selective cation-channels that function in mechanosensation, osmoregulation and replenishment of intracellular calcium stores. This TRPV family includes at least five members, three of which are expressed in DRG neurons. One of these, VR1 (TRPV2), is also gated by heat, but has a higher threshold than VR1 (52°C instead of 43°C) and is not co-expressed with VR1. Recent experiments have implied that VR1 expression does not correlate with the heat-sensitive neurons in VR1 knockout mice, suggesting the existence of yet another heat-sensing channel.

[0217] Public and Celera databases for VR1-related TRP channels are searched by constructing a Hidden Markov Model (HMM) of the VR1 and VR1L protein sequences from different mammalian species. With this model, the 6-frame translation of human sequence is queried and has identified multiple new putative exons with a great degree of sequence similarity to the ankyrin and transmembrane domains of VR1. These exons map to two genes, one of which is TRPV4, as described, e.g., in Liedtke et al., *Cell*, 103:525-35 (2000); and Struemann et al., *supra*. The other novel gene is known as TRPV3.

[0218] The full-length sequence of mouse TRPV3 is derived from a combination of exon-prediction software, PCR and RACE amplification from newborn mouse DRG and skin cDNA. For PCR cloning, primers (5'-TGACATGATCTGCTGAGAGAGT-3'

(SEQ ID NO: 19) and 5'-ACGAGGACGGCAGGATTTCTT-3' (SEQ ID NO: 20)) are designed from the HMM sequences for TRPV3 as a result of Blast hits to the ankyrin and transmembrane domains and used to amplify a 699-nucleotide fragment of TRPV3 from newborn DRG cDNA. From this initial fragment, Rapid Amplification of cDNA Ends (RACE) PCR (Clontech) is used to obtain the 5' and 3' ends of TRPV3 from mouse newborn skin and DRG cDNA. In order to characterize the genomic locus of VR1 and TRPV3, primers are designed from predicted HMM TRPV3 exon sequences and used to screen a genomic BAC Mouse (RPCL22) library (Roswell Park Cancer Institute). Primers utilized are shown in Table 1. Additionally, mouse VR1 BACs are identified by hybridizing a 320 bp probe spanning the mouse VR1 ankyrin region to the same BAC library. Positive BAC clones are further characterized by restriction digest mapping, pulse field gel electrophoresis, and Southern blotting as previously described using probes specific to the 5' and 3' ends of the VR1 and TRPV3 genes. BAC clones positive for TRPV3 included 513, BAC clones that were positive for both VR1 and TRPV3 included 9e22, 27114, 82e1 and 112g17. BACs positive for VR1 included 137N13, 137O13, 234I23, 246D9 and 285G11.

Table 1: TRPV3 Primers

	5' RACE	SEQ ID NO:
AP40	CAGCGTATGCAGAGGCTCCAGGCTCAG	21
AP4	TTGAAGTCTCAGCCACCGTCACCA	22
Mvr4ANK	CACCAAGCGGTGCAGGATGT	23
AP105 RACE-rev	tggtctcagcaggagcagcaga	24
AP110R (nested)	CCITCTATCTCCAGGAGAAAGTGTGC	25
ap113r (race)	GTCACCAGCGCGTCAGAGATGTTGT	26
ap36	AGGCCCATACGCCAGTCCGTGAAC	27
ap33R	CATGCCATAGACTGGAAGCC	28
ap71	GATGGCATGTTCAAGCGCTGTCTGC	29
3' RACE		
AP37	GCTGCCAAGATGGCAAGGCTGAGA	30
Ap31	CCTGGGCTGGGCGAACAATGCTCTA	31
TM6VR4RACE	GCGCCAGATGCGTTACATTTCTTTGGA	32
Primers to amplify partial and/or full-length TRPV transcript		
mVR4-F	TGACATGATCTGCTGAGAGAGTG	33
mVR4-R	ACGAGGACGGCGAGGTAITTCIT	34

WO 02/101045	PCT/EP02/06520	PCT/EP02/06520
AP72 F	TCCAAGCTGTGCTTGATA	35
AP73R	CTTGAGCATGTAGTTTCACAAAA	36
AP74R	GTGTTTCCATTCCGTCCAC	37
AP75R	CGACGTTTCTGGGAAATTCAT	38
AP76R	CTTGAGCATGTAGTTTCACAAAA	39
AP77F	TCCTCCTCCTCAACATGCTC	40
AP78R	TGGAAATCAAAACAGTATTCAATG	41
AP79F	CTCTTCAAGCTCACCATAGGC	42
AP80R	CGACGTTTCTGGGAAATTCAT	43
AP81R	GTGTTTCCATTCCGTCCAC	44
AP82R	CCCTCTGTATCCGCAGACAC	45
AP83F	ACTCCAGCCTGGGTGACA	46
AP84R	ATGCTCTCCAGCTCCAGTT	47
AP85R	AGGAGGACGAAGGTGAGGAT	48
AP86F	AGCCTCAGGCTCTGAAAGTGA	49
AP87R	GCCAGATGCGTTCACCTTCT	50
AP88R	GGCAAAATTTCTTCCATTTCG	51
AP89R	AGATCGGTTTCGCTCTCCTT	52
AP102F	TGCACACTTCTTCTCTGGAGAT	53
AP103F	TTCTCTCATGCACAAAGTGAC	54
AP104F	TCTTCTGGAGATAGAAGGATT	55
AP106R	CGATGATTTCCAGCACACAG	56
AP107F	CTCACCAAATGTAGACACACAGAC	57
AP108F	TACCAGCATGAAGGCTTCTATTT	58
AP109R	ATAAGCACTGCTGTGATGTCTCC	59
AP111R	GTCAGCTTGTGCATGAGGAA	60
AP112F	TGACAGAGACCCCATCCCAACA	61
AP114F	CTCTTGATATGGCTTCTGG	62
AP115F	GAGAAGGATGGGTGAGCTG	63
AP116R	CCTTCTCCAGAGTCCACAG	64
AP117F	AGCAGGCAGGAAAATGAGAG	65
AP118R	CCAAAAGATGGTCCAGAAAGC	66
AP115F	CTCTTGATATGGCTTCTGG	67
AP116F	AACTGTGATGACATGGACTCTCCCCAG	68

WO 02/101045	PCT/EP02/06520	PCT/EP02/06520
AP118F	AACTGTGATGACATGGACTC	69
AP119F	CAGGATGATGTGACAGAGACCCCATC	70
AP128F	ATGATCCTGCTGAGGAGTGG	71
AP129R	AGGATGACACACAGGCCCATAC	72
AP130F	ATCCTCACCTTCGCTCCTCT	73
AP131R	CATTCCGTCCACTTCACCTC	74
AP204R (3'UTR)	TGGTTTGTGCTGTGTTCTCTG	75
AP205R	(POLYA)CATGTAAATCAACGCAGAAAGTCA	76

[0219] Several murine ESTs from skin tissues contain 3' UTR TRPV3 sequence (BB148735, BB148088, BB151430 and AI644701), and recently the human TRPV3 sequence has been annotated (see GI: 185877, 18587705 and Peng et al., *Genomics*, 76:99-109 (2001)).

[0220] As predicted from the nucleotide sequence, TRPV3 is composed of 791 amino acid residues. The overall sequence of mouse TRPV3 has 43% identity to TRPV1 (VR1) and TRPV4; 41% to TRPV2 (VRL1); and 20% to TRPV5 (ECAC) and TRPV6 (see Figure 2C). TRPV3 has four, instead of the usual three, predicted N-terminal ankyrin domains that are thought to be involved in protein-protein interactions, TM6 domains and a pore loop region between the last two membrane spanning regions. Two coiled-coil domains N-terminus to the ankyrin domains in TRPV3 are also identified (see Figure 2F). Coiled-coil domains are implicated in oligomerization of GABA-B channels, and have been previously reported to be present in some TRP channels, but not for TRPVs. Further examination shows that VR1, but not the other members of the TRPV family, also has putative coiled-coil domains in the same N-terminal location. Phylogenetic analysis illustrates that TRPV3 is indeed a member of the OTRP/TRPV sub-family, which is part of the larger TRP ion channel family (see Figure 2A). The same BAC genomic clone in the public database contains the sequence of TRPV3 and VR1. Both genes map to human chromosome 17p13 and mouse chromosome 11B4. Mapping analysis of these BAC clones, and later the assembled human and mouse genome sequences reveals the distance between the two genes to be about 10 kb (see Figure 2B). This suggests that TRPV3 and VR1 are derived from a single duplication event.

EXAMPLE 2

Localization of TRPV3 Expression*A. Northern blot analysis*

[0221] For Northern blot analyses approximately 3 µg of polyA⁺ RNA extracted from adult mouse and newborn tissue are electrophoresed on 1% glyoxal gels, transferred and hybridized at high-stringency with a ³²P labeled probe representing the entire full-length TRPV3 sequence. Commercial Northern blots (Clontech) are hybridized with the same TRPV3 full-length probe. For human skin specific expression, Northern blots are prepared from 20 µg of total RNA from primary keratinocytes and cell lines CRL-2309 and CRL-2404 (ATCC) or from 2 µg of polyA⁺ adult and fetal skin RNA (Stratagene). Blots are hybridized with a probe corresponding to the ankyrin 1-TM2 region of the TRPV3 human sequence. For VR1 hybridizations, a probe corresponding to nucleotides 60-605, encoding the amino terminus of rat VR1 are used on mouse blots. The entire coding sequence of human VR1 are used as a probe on human Northern blots.

[0222] As stated above, to determine the overall tissue distribution of TRPV3, the full-length mouse TRPV3 sequence is used as a probe for Northern blot analysis. No TRPV3 expression is detected using commercial Northern blots. Blots from adult rat are then used that include tissues relevant to somatic sensation, including DRG, spinal cord and different sources of skin. A mRNA of approximately 6.5 kb is present in tissues derived from skin but not in DRGs. Probing the same adult blot with a TRPV1-specific probe confirms its strong expression in DRG while demonstrating a lack of expression in skin tissues. Northern blot analysis of human adult and fetal skin also shows expression of TRPV3. Cultured primary mouse keratinocytes as well as several epidermal cell lines do not show any TRPV3 expression by Northern blots. These finding suggest that TRPV3 expression may get down regulated after tissue dissociation and long-term culture. Northern blots from newborn and adult mice that include tissues relevant for somatic sensation, including DRG, spinal cord and different sources in skin also show TRPV3 expression in skin tissues with weak expression in the DRG.

B. In situ hybridization

[0223] For *in situ* hybridizations, newborn and adult tissues are dissected, fixed in 4% paraformaldehyde in PBS, cryoprotected and frozen in liquid nitrogen in OCT mounting

medium. Cryostat sections (10 µm) are processed and probed with either a digoxigenin cRNA probe or a ³⁵S-labeled probe generated by *in vitro* transcription as described in Wilkinson, in *Essential Developmental Biology, A Practical Approach*, C. Stern, P. Holland, eds, Oxford Univ. Press, NY, pp. 258-263 (1993). Two mouse TRPV3-specific antisense riboprobes are used, one corresponding to nucleotides 235-1020 encoding the amino terminus and the other spanning nucleotides 980-1675 corresponding to the region between the third ankyrin and TM4 domains.

[0224] Digoxigenin-labeled probes show specific expression in specialized skin tissues, such as hair follicles in both newborn and adult mice. Expression in epidermis is difficult to assess, because of high background observed in this tissue with the sense probe. To circumvent this problem, and to gain more sensitivity, ³⁵S-radioactive *in situ* hybridizations are carried out on cross-sections of newborn mice. Clear expression is detected in the epidermis and hair follicles. No significant expression is detected in DRGs.

C. Immunohistochemical staining assays

[0225] For immunohistochemistry, rabbits are immunized (AnimalPharm Services, Heidelberg, CA) with KLH conjugated peptide corresponding to either the N-terminus of mouse TRPV3 (CDDMDSPQSPQDDVTETPSN (SEQ ID NO: 77)) or a C-terminus peptide (KIQDSSRSNSKTTL (SEQ ID NO: 78)). Affinity purified antiserum recognizes a band of relative molecular mass ~85 kDa in whole-cell extracts prepared from CHO cells stably transfected with mouse TRPV3 (not shown). For peptide competition, diluted antibody solutions (1:5000) of TRPV3 are pre-incubated (room temperature, 2 hours) with TRPV3 antigenic peptide (9 µg/mL⁻¹) prior to incubation with tissue sections. Immunofluorescence are performed on fixed frozen and paraffin sections using rabbit anti-TRPV3 (1:5000), pan cytokeratin (Abcam) cyokeratin (1:300, Abcam), cyokeratin 10 (K8.60, Sigma), pan-basal Cyokeratin (Abcam), PGP9.5 (Abcam) followed by FITC-labeled goat anti-rabbit (10 µg/mL⁻¹) and Cy-3-labeled donkey anti-mouse (Jackson ImmunoResearch) antibodies.

[0226] Using polyclonal antibodies produced against TRPV3 peptides from either the N-terminus or the C-terminus, intense TRPV3 immunoreactivity is observed in most keratinocytes at the epidermal layer and in hair follicles from newborn and adult rodent tissues. In the epidermis, staining is absent in the outermost layers (stratum corneum and

lucidum) as well as the basement membrane. In hair follicles, expression is localized to the outer root sheath and absent from the matrix cells, inner root sheath and sebaceous glands. Developmentally, expression in hair follicles increases from newborn to adult stages. High magnification of these images indicates staining in the cytoplasm and at high levels in the plasma membrane.

[0227] Coexpression with various keratinocyte-specific markers shows that TRPV3 is expressed in the basal keratinocytes, which *in vitro* require low calcium concentrations to maintain their undifferentiated state, as well as in some of the more differentiated suprabasal layers of the epidermis. Temperature-sensing neurons are thought to terminate as free nerve endings mainly at the level of dermis, but some processes do extend into the epidermis (see Hilliges et al., *supra*; and Cauna, *supra*. Cutaneous termini can be labeled with the immunohistochemical marker protein gene product 9.5 (PGP 9.5), and it is observed that these epidermal endings indeed co-localize with TRPV3.

D. GFP-fusion constructs

[0228] The full-length mouse TRPV3 is amplified and subcloned into pcDNA3.1/CT-GFP-TOPO (Invitrogen). *In vitro* transcription/translation (TnT System, Promega) confirms the integrity of the constructs. Cells are viewed live or fixed in 4% paraformaldehyde 48-72 hours after transfection, counterstained with propidium iodide and mounted in Slowfade (Molecular probes).

[0229] Confocal fluorescence microscopy on cells transiently transfected with a C-terminally GFP-tagged TRPV3 protein construct also finds the protein mainly localized at the plasma membrane. This pattern of expression at the cell membrane is consistent with TRPV3 having a role as an ion channel. In sum, the expression analysis suggests that TRPV3 is most prominently expressed in plasma membrane of keratinocytes in both rodents and humans.

EXAMPLE 3

Activation of TRPV3 Protein by Heat

A. Effect of heat, capsazepine and ruthenium red upon conductance

[0230] Given the high degree of homology of TRPV3 to TRPV family members, TRPV3 is tested to determine whether it responds to stimuli known to activate other closely-

related family members. Accordingly, the effects of heat upon TRPV3 activity in mediating conductance are examined using whole-cell patch-clamp analysis of transfected CHO cell lines expressing TRPV3.

[0231] Mouse TRPV3 and rat TRPV1 cDNA are subcloned into pcDNA5 (Invitrogen) and transfected into CHO-K1/FRT cells using Fugene 6 (Roche). The transfected cells are selected by growth in MEM medium containing 200 µg/mL hygromycin (Gibco BRL). Populations are frozen at early passages and these stocks are used for further studies. Stable clones that express the mRNAs are identified by Northern blot analysis as well as Southern blotting to confirm integration site. Long-term cultures are subsequently maintained at 33°C.

[0232] TRPV3 expressing CHO cells are assayed electrophysiologically using whole cell voltage clamped techniques. Currents are recorded via pCLAMP8 suite of software via an Axopatch 200A and filtered at 5 kHz. Series-resistance compensation for all experiments is 80% using 2-5 MΩ resistance, fire-polished pipettes. Unless stated, the holding potential for most experiments is -60 mV, apart from the current-voltage relationship studies (2 second ramp from -100 to +80 mV). Cells are normally bathed in a medium containing (mM): NaCl, 140; KCl, 5; Glucose, 10; HEPES, 10; CaCl₂, 2; MgCl₂, 1; titrated to pH 7.4 with NaOH, apart from the monovalent permeability studies, when NaCl is replaced by equimolar KCl or CsCl with the omission of KCl, 5 mM. For the divalent permeability studies, the solutions either contain 1 mM Ca²⁺ or Mg²⁺ and (mM) NaCl, 100; Glucose, 10; Hepes, 10; sucrose, 80 or 30 mM test ion, in the above solution minus sucrose. The experiments in calcium free media have no added CaCl₂ with the addition of 100 µM EGTA. Pipette solution is always (mM) CsCl, 140; CsCl, 140; CaCl₂, 1; EGTA, 10; HEPES, 10; MgATP, 2; titrated to pH 7.4 with CsOH. For the permeability, ratios for the monovalent cations relative to Na (P_X/P_{Na}) are calculated as follows:

$$P_X/P_{Na} = E_{\text{shift}} = \{RT/F\} \log (P_X/P_{Na} [X]_o / [Na]_o)$$

where F is Faraday's constant, R is the universal gas constant, and T is absolute temperature. For the divalent ions, P_{Ca} or P_{Mg}/P_{Na} is calculated as follows:

$$E_{\text{shift}} = \{RT/F\} \log \{([Na]_o + 4B' [X]_o / 2)\} / \{([Na]_o + 4B' [X]_o / 2)\}$$

where $B' = P'_X/P_{Na}$ and $P'_X = P_X/(1 + e^{E_{\text{shift}}/RT})$ and $[X]_o$ and $[X]_o / 2$ refer to the two different concentrations of the divalent ion tested.

[0233] The results from transfected cells assayed electrophysiologically via whole cell voltage clamped techniques are described below. Capsaicin (1 μ M), an activator of TRPV1, does not evoke a response in TRPV3-expressing cells. Similarly no current responses are seen when TRPV3-expressing cells are challenged with a hypo-osmotic solution containing 70 mM NaCl or with low pH (5.4). However, raising the temperature of superfused external solution from room temperature to 45°C evokes currents in TRPV3 expressing cells. Analysis of currents evoked by temperature ramps from ~15°C to ~48°C (see Figure 3A) shows that little current is elicited until temperatures rise above ~33°C and that the current continues to increase in the noxious temperature range (>42°C). With these findings, TRPV3-expressing cells are subsequently maintained at 33°C to avoid constitutive activation. The current amplitude is influenced by the presence or absence of Ca^{2+} in the external medium, with reduced current amplitudes in the presence of 2 mM Ca^{2+} after a prior challenge in Ca^{2+} -free solution (see Figure 3B). This finding is reminiscent of the channel properties of TRPV5 and TRPV6 (see Nilius et al., *J. Physiol.*, 527:229-248 (2000)). As shown in Figure 3C, the heat evoked current in TRPV3-expressing CHO cells increases exponentially at temperatures above 35°C with an e-fold increase per $5.29 \pm 0.35^\circ\text{C}$ ($n=12$), corresponding to a mean Q_{10} of 6.62. This temperature dependence is considerably greater than that seen for most ion channel currents, which typically have Q_{10} values in the range 1.5-2.0, but is less than the values noted for TRPV1 (VR1, $Q_{10} = 17.8$) (see Vyklicky et al., *J. Physiol.*, 517:181-192 (1999)). In some cells it is difficult to see a sharp threshold temperature. However, measurable temperature dependent currents below 30°C show an e-fold increase for a $22.72 \pm 3.31^\circ\text{C}$ ($n=12$) increase in temperature ($Q_{10} = 1.69$).

[0234] The elevated temperature evoked currents, in TRPV3-expressing cells, shows a pronounced outward rectification (see Figure 3D) with a reversal potential in the standard recording solution of -1.22 ± 1 mV. Reducing the NaCl in the external solution to 70 mM (from 140 mM) shifts the reversal potential by -19mV as expected for a cation selective conductance (shift = -17.5 mV). Differences in reversal potentials are also used to determine the ionic selectivity of TRPV3 channels. In simplified external solutions, the reversal potentials of the heat activated currents are very similar when NaCl ($E_{\text{rev}} = -1.22 \pm 1.08$ mV, $n=5$) is replaced with either KCl ($E_{\text{rev}} = -0.40 \pm 0.77$ mV, $n=6$) or CsCl ($E_{\text{rev}} = -1.14 \pm 0.53$ mV, $n=6$), which yields relative permeability ratios $P_{\text{K}}/P_{\text{Na}}$ and $P_{\text{Cs}}/P_{\text{Na}}$ close to 1 (see Funayama et al., *Brain Res. Mol. Brain Res.*, 43:259-266 (1996)). The relative

permeability of Ca^{2+} and Mg^{2+} are estimated from the shift in reversal potentials when their concentrations are raised from 1 mM to 30 mM in a 100 mM NaCl solution containing the divalent cation under investigation. The reversal potential shifts (from -9.1 +1.40 mV to +1.29 + 0.38 mV for Ca^{2+} and from -8.41 \pm 0.50 mV to +10.34 \pm 2.38 mV for Mg^{2+}) correspond to $P_{\text{Ca}}/P_{\text{Na}} = 2.57$ and $P_{\text{Mg}}/P_{\text{Na}} = 2.18$. These data show that TRPV3 is a non-selective cation channel that discriminates poorly between the tested monovalent cations and has significant divalent cation permeability.

[0235] Heat activation of TRPV3 shows a marked sensitization with repeated heat stimulation. This is studied at a steady membrane potential of -60 mV and with voltage ramps. The first response to a step increase from room temperature to ~48°C is often very small, but the current response grew with repeated heat steps (see Figure 4A). Sensitization to heat has also been observed for TRPV1 and TRPV4 (see Caterina et al., *supra* and Jordt et al., *Cell*, 108:421-430 (2002)). Application of voltage ramps shows that sensitization is associated with an increase in outward rectification (see Figure 4B). A protocol of repeated temperature challenges is used to investigate if antagonists of TRPV1 (VR1) are inhibitors of TRPV3. Under normal conditions, a heat challenge delivered 2 minutes after 4-5 sensitizing heat steps evokes a current that is 1.57 ± 0.25 ($n=4$) times the amplitude of the preceding response (see Figure 4C). Application of 10 μ M capsaizepine, a competitive capsaicin antagonist at TRPV1, for 2 minutes prior to the test heat challenge does not reduce the current amplitude (2.31 ± 0.36 times the amplitude of the preceding response, $n=4$). In contrast, a similar exposure to 1 μ M ruthenium red, a non-competitive inhibitor of other TRPV channels, reduces the relative amplitude of the heat response to 0.34 ± 0.03 , $n=5$ (see Figure 4D). Taken together, these results indicate that TRPV3 is a cation permeable channel activated by warm and hot temperatures and has channel properties reminiscent of other TRPV channels.

EXAMPLE 4

Gene Expression Analysis of TRPV3 in the Rat Chung Model
[0236] These studies discussed below measure relative levels of RNA expression for TRPV3 in the Chung neuropathic pain model using RT-PCR.

A. Spinal nerve ligation (Chung) model

[0237] This model is established according to the methods described by Kim and Chung, *supra*, 1992. Rats are anesthetized and placed into a prone position and an incision made to the left of the spine at the L4-S2 level. A deep dissection through the paraspinal muscles and separation of the muscles from the spinal processes at the L4-S2 level will reveal part of the sciatic nerve as it branches to form the L4, L5 and L6 spinal nerves. The L6 transverse process is carefully removed with a small rongeur enabling visualization of these spinal nerves. The L5 spinal nerve is isolated and tightly ligated with 7-0 silk suture. The wound is closed with a single muscle suture (6-0 silk) and one or two skin closure clips and dusted with antibiotic powder. In sham animals the L5 nerve is exposed as before but not ligated and the wound closed as before.

[0238] Male Wistar rats (120-140 g) are used for each procedure. Mechanical hyperalgesia is assessed by measuring paw withdrawal thresholds of both hindpaws to an increasing pressure stimulus using an Analgesymeter (Ugo-Basile, Milan). Mechanical allodynia is assessed by measuring withdrawal thresholds to non-noxious mechanical stimuli applied with von Frey hairs to the plantar surface of both hindpaws. Thermal hyperalgesia is assessed by measuring withdrawal latencies to a noxious thermal stimulus applied to the underside of each hindpaw. With all models, mechanical hyperalgesia and allodynia and thermal hyperalgesia develop within 1-3 days following surgery and persist for at least 50 days. Drugs may be applied before and after surgery to assess their effect on the development of hyperalgesia, or approximately 14 days following surgery to determine their ability to reverse established hyperalgesia.

B. RT-PCR mRNA analysis

[0239] One microgram of total RNA samples from the Chung model (L4 and L5 DRG) and sham-operated animals are used for first-strand cDNA synthesis using 50 pmol of oligo (dt) 24 primer in a 20 μ L total reaction with 200 units Superscript II (LTI). The cDNA is then diluted to 100 μ L with Tris-EDTA buffer (10 mM TrisCl, pH 8.0 and 1 mM EDTA). Three μ L of the diluted cDNA is used to amplify the message for TRPV3 with gene-specific primers (sequences in 5' to 3' orientation: TRPV3 forward primer, CTCATGCACAAGCTGACAGCCT (SEQ ID NO: 79); TRPV3 reverse primer, AGGCTCTCTCCGTGTACTCAGCGTTG (SEQ ID NO: 80)) in a 15 μ L PCR reaction

using NotStart Taq DNA polymerase (Qiagen) for 25-38 cycles. Neuropeptide Y (NPY) is used as positive control.

[0240] For normalization 1 μ L of the diluted cDNA is used to amplify actin using the following primers:

5' actin primer: ATC TGG CAC CAC ACC TTC TAC AA (SEQ ID NO: 81)

3' actin primer: GCC AGC CAG GTC CAG ACG CA (SEQ ID NO: 82)

[0241] A portion of the samples are then analyzed on a 4-20 TBE Criterion polyacrylamide gel (BioRad), stained with SYBR GREEN I (Molecular Probes) and visualized on a Phosphorimager.

[0242] Figure 1A shows the average fold regulation of TRPV3 (VRLx) in L4 and L5 DRG neurons from the Chung model from three independent experiments. As shown in Figure 1A the positive control, NPY and TRPV3 message are elevated in the injured DRG relative to sham and non-ligated DRGs.

EXAMPLE 5

15 Identification of TRPV4

[0243] Primers are designed to amplify distinct regions of the candidate genes that had been identified through the computer model. Based on the human sequence obtained, PCR primers are designed to also amplify the mouse homologue of TRPV4 (mTRPV4)

(TRPV4 forward: CTCATGCACAAGCTGACAGCCT (SEQ ID NO: 83); TRP4 reverse:

20 AGGCTCTCTCCGTGTACTCAGCGTTG (SEQ ID NO: 84)). These PCR products are subsequently sequenced and the mouse EST database is searched using these sequences.

One EST clone (ID No. AI510567) is identified and obtained from the IMAGE consortium. The EST is further characterized and found to contain a ~2.4 kb insert which is sequenced.

Primers are designed from this sequence and used to obtain the full length cDNA using the RACE protocol (Clontech). Both 5' and 3' RACE products are obtained and sequenced.

25 This approach results in the amplification of the full length cDNA of mTRPV4 from mouse kidney and DRG cDNA using primers designed from the very 5' and 3' end of the RACE products. All primers utilized in the characterization of mTRPV4 are shown in Table 2. A novel full length cDNA of ~3.2 kb is identified, which includes an open-reading frame of ~2.5 kb, a 5' UTR consisting of ~145 bp and a 3' UTR encompassing ~400-500 nucleotides. The gene encodes a 3.4 kb transcript that contains three ankyrin-repeat regions and TM6

domains. The protein sequence includes ~1000 amino acids and is set forth in SEQ ID NO: 14. Clustal W alignments to the rat VR (GenBank Accession No. AF029310) reveals 34% identity and 64% similarity to VR1 in the region spanning the Ank2 through the TM4 region.

5

Table 2: TRPV4 Primers

Primers used for RACE		SEQ ID NO:
3' RACE	CCCTGGGCTGGCGAACAATGCTCTA	85
VR3RACE5'	CTTGGCAGCCATCATGAGAGCGGAA	86
Primers to amplify partial/full length TRPV4		
AP19	GCAGTGGTAAACAACGCAGAG	87
AP20	AGGTCAGATCTGTGGCAGGT	88
AP21	CGTGAAGGTGACAGATGAGGA	89
AP32	CCAGTATGGCAGATCCTGGT	90
AP25	ATGGCAGATCCTGTGATG	91
AP26	CCCAGGCACTACTGAGGACT	92
AP27	AGGGCTACGCTCCCAAGT	93
AP28	GTGCTGGCTTAGGTGACTCC	94
AP22	TGAACCTGGCAGACAGATGC	95

[0244] A combination of RT-PCR and Northern blot analyses are utilized to characterize expression of TRPV4. Total RNA is prepared from adult mouse kidney, newborn DRG and adult trigeminal tissue. RT-PCR is carried out using cDNA prepared from these three mouse tissues and primers within the ankyrin and the TM domain of mTRPV4. The expected 403 bp product is observed in all three tissues. This PCR product also serves as a probe on Northern blots (Clontech MTN blots). The expected 3.4 kb transcript is observed in kidney and other tissues.

10 [0245] The genomic structure of hTRPV4 is predicted from the high throughput genomic sequence database (GenBank Accession No. AC007834). HV3R encompasses ~17 exons. A comparison of the amino acid sequence of the rat VR1 sequence (GenBank Accession No. AF029310) and the mouse VR3 protein reveals 34% identity and 64% similarity in the sequence spanning the ankyrin 2 region and the TM4 domain. The nucleotide and amino acid sequences of hTRPV4 are shown in SEQ ID NO: 16 and SEQ ID NO: 17, respectively.

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EXAMPLE 6

Gene Expression Analysis of TRPV4 in the Rat Chung Model

[0246] These studies discussed below measure relative levels of RNA expression for TRPV4 in the Chung neuropathic pain model using RT-PCR.

5 A. Spinal nerve ligation (Chung) model

[0247] This model is established according to the methods described by Kim and Chung, *supra*, and is described in Example 4.

B. RT-PCR mRNA analysis

[0248] One microgram of total RNA samples from the Chung model (L4 and L5 DRG) and sham-operated animals are used for first-strand cDNA synthesis using 50 pmol of oligo (dt) 24 primer in a 20 μ L total reaction with 200 units Superscript II (LIT). The cDNA is then diluted to 100 μ L with Tris-EDTA buffer (10 mM TrisCl, pH 8.0 and 1 mM EDTA). Three μ L of the diluted cDNA is used to amplify the message for TRPV4 with gene-specific primers (Sequences in 5' to 3' orientation: TRPV4 forward primer, 99

15. TGAGGATGACATAGGTGATGAG 120 (SEQ ID NO: 96), TRPV4 reverse primer, 255 CCAAGGACAAAAGGACTGC 236 (SEQ ID NO: 97)) in a 15 μ L PCR reaction using NotStart Taq DNA polymerase (Qiagen) for 25-38 cycles. NPY is used as positive control.

[0249] For normalization 1 μ L of the diluted cDNA is used to amplify actin using the following primers:

20 5'actin primer: ATC TGG CAC CAC ACC TTC TAC AA (SEQ ID NO: 81)

3'actin primer: GCC AGC CAG GTC CAG ACG CA (SEQ ID NO: 82)

[0250] A portion of the samples are then analyzed on a 4-20 TBE Criterion polyacrylamide gel (BioRad), stained with SYBR GREEN I (Molecular Probes) and visualized on a Phosphorimager.

25 [0251] First-strand cDNA from the Chung model (50 days post-ligation) is normalized using a house-keeping gene; beta-actin. Figures 1A and 1B shows the expression of TRPV4 and NPY in the Chung Model (50- and 28-day post-ligation, respectively). The positive control, NPY and TRPV4 message are elevated in the injured DRG relative to sham and non-ligated DRGs. Accordingly, TRPV4 serves as a target for

30 neuropathic pain.

EXAMPLE 7

Identification of VR TRPM8

[0252] To identify novel TRP channels, genomic DNA databases are searched by constructing a HMM from the known TRP protein sequences of different mammalian species. With this model, the 6-frame translation of all available human sequences is queried and identifies multiple novel putative exons with similarity to the TM4 and TM6 domains of VR1. A fragment of the mouse homologue of one novel TRP channel is amplified by RT-PCR from mouse DRG RNA. Full-length sequence of this gene is derived from a combination of exon-prediction software, PCR and RACE amplification from

5 newborn mouse DRGs.

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[0253] For PCR cloning, primers 163f (5'-CAAGTTTGTCCGGCTCTTTC (SEQ ID NO: 98)) and 164r (5'-AACTGTCTGGAGCTGGCAGT (SEQ ID NO: 99)) are designed from the HMM sequences for TRPM8 as a result of blast hits and used to amplify a 699-nucleotide fragment of TRPM8 from newborn DRG cDNA. From this initial sequence and exon prediction programs, RACE PCR (Clontech) is used to obtain the 5' and 3' ends of TRPM8 from mouse newborn DRG cDNA following the manufacturer's protocol. Primers used in these experiments are shown in Table 3.

15

Table 3: Primers to Amplify Mouse TRPM8 cDNA

	Putative trp candidate 2KMHMRSR44-MOD CELEERA HUMAN CONTIG	SEQ ID NO:
FOR MOUSE:		
Probes designed for <i>in situ</i> hybrid analysis		
AP163F	CAAGTTGTCCGCTCTTTC	100
AP164R	ACTGCCAGCTCCAGACAGTT	101
Rapid amplification of cDNA ends (RACE)		
5' RACE primers		
5' RACE (nested)	cctgatgctgctctggcgaata	102
5' RACE	CCTTGCCCTTCTTCCCAAGATCTCAA	103
AP220 5' RACE	GCAAAGTTTGTGGCTCCACCCGTC	104
AP2215' RACE (nested)	GCCAAGTCTGGGTCAAGCAATTCGTA	105
3' RACE primers		
3' RACE 1	TTCAAGAGGTCATGTTACGGCTCTCA	106
3' RACE 1 (nested)	GTACCGGAACCTGCAGATCGCCAAGA	107
AP218 3' RACE TRPXII	GCAAAGATCCCTTGTGTGGTGTGGA	108
AP219 3' (nested)	CAGCTGGTGGAGGTGGAGGATGTT	109
3' RACE #3	CGGAACCTGCAGATCGCCAAGAAGT	110
3' RACE primer in TM5 region of TRPM8		
AP225	GCGTGGCCAGACAGGGGATCCTAAG	111
3' REVERSE primer in TM5 region of TRPM8		
AP226	CCACACAGCAAAAGAGAAACA	112
To amplify longer piece of mouse TRPM8		
216F	GGAGCCCGCAGAATGTAAT	113
Primers used for Northern probe		
Amplifies around 1.2 KB band		
AP258	TCTCATTTGGCCTCATTTTCTG	114
AP247	ATATGAGACCCGAGCAGTGG	115

[0254] The protein TRPM8, has been named following the nomenclature suggested in Clapham et al., *Cell*, 108:595-598 (2001). Several human ESTs, many of which have been isolated from various cancer tissues, contain fragments of TRPM8 (Genbank GI Nos. 8750489, 9149390, 9335992 and 2223353).

[0255] Translation of the nucleotide sequence of TRPM8 predicts a protein composed of 1104 amino acid residues (see SEQ ID NO: 8). The overall sequence of mouse

TRPM8 is 93% identical to that of the human gene (see Figure 6A). Its closest relative is TRPM2 (42% identity) (see Figures 6A and 6B). TRPM8 belongs to the "long" or Melastatin subfamily of TRP channels, a group of TRPs characterized by their lack of ankyrin domains in the N-terminus. TRP channels are predicted to contain TM6 domains, although at least one is predicted to have seven membrane-spanning domains (see Nagamine et al., *Genomics*, 54:124-131 (1998)). A Kyte-Doolittle plot suggests the presence of eight distinct hydrophobic peaks in TRPM8 sequence, which could represent six to eight predicted transmembrane domains. Overall, the predicted transmembrane domains are within amino acids 695-1024 of TRPM8. Outside of this region, the only predicted secondary structures are coiled-coil domains present both in the N- and C-terminal portion of the protein (data not shown) (see Buthard et al., *Trends Cell. Biol.*, 11:82-88 (2001)). Coiled-coil domains are implicated in oligomerization of GABA-B channels, and have been previously predicted in some TRP channels (see Funayama et al., *supra*, and Margalea-Mitrovic et al., *Neuron*, 27:97-106 (2000)).

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EXAMPLE 8

Localization of TRPM8 expressionA. Northern blot analysis

[0256] Northern blots are made as followed: Total RNA are purified from mouse newborn and adult tissues using TRIzol LS (Invitrogen/Gibco Life technologies), followed by polyA⁺ purification with Oligotex (Qiagen) according to the manufacturer's protocols. Approximately 3 mg of sample are electrophoresed on 1% glyoxal gels, transferred and hybridized at high-stringency with a ³²P-labeled probe representing nucleotides 1410-1980 of the mouse full-length TRPM8 sequence. Commercial Northern blots (Clontech) are hybridized with the same TRPM8 probe. Blots are hybridized for 3 hours at 68°C in ExpressHyb hybridization solution (Clontech) and washed according to the manufacturer's high-stringency washing protocol and exposed to a phosphorimager screen for 1-3 days.

[0257] The results from this analysis are described below. No TRPM8 expression is detected using commercial Northern blots. Blots from newborn and adult mice are used that include tissues relevant for somatic sensation, including DRG, spinal cord and different

25

sources of skin. One mRNA species of approximately 6.3 kb is present predominantly in DRGs.

B. *In situ* hybridization

[0258] For *in situ* hybridizations, newborn and adult tissues are dissected, fixed in 4% paraformaldehyde in PBS, cryoprotected and frozen in liquid nitrogen in OCT mounting medium. Cryostat sections (10 μ m) are processed and hybridized with a digoxigenin cRNA probe generated by *in vitro* transcription (Roche Biochemicals). The mouse TRPM8 mRNA-specific antisense riboprobe corresponds to nucleotides 1410-1980 of the mTRPM8 sequence. Fluorescence detection and double-labeling experiments are carried out with the tyramide signal amplification kit (TSA; NEN) essentially as previously described (see Dong et al., *Cell*, 106:619-632 (2001)).

[0259] Digoxigenin-labeled probes show specific expression in DRG and

trigeminal ganglia (cranial sensory neurons innervating the mouth and jaw) in newborn and adult mouse, but not in day 13 embryos. TRPM8 expression is restricted to approximately

5-10% of adult DRG neurons. The average size of the neurons positive for TRPM8 is 18 ± 3.1 μ m (mean \pm standard deviation, $n=69$), and can be classified as small-diameter c-fiber-containing neurons, which in mouse are defined as smaller than 25 μ m. TRPM8 is not expressed in heavily-myelinated neurons marked by Neurofilament (NF) antibodies, which correlates well with TRPM8 expression in small-sized neurons. TRPM8⁺ neurons thus appear to belong to a subset of nociceptive or thermoreceptive neurons that express trkA, an NGF receptor, during development (see Huang and Reichardt, *Ann. Rev. Neurosci.*, 24:677-736 (2001)). In the absence of NGF or trkA, DRG neurons that normally express this

receptor die through apoptosis during embryonic development (Huang and Reichardt, *supra*). To prove that TRPM8 is expressed in trkA-dependent neurons, TRPM8 expression is evaluated in DRGs from newborn trkA-null mice. The expression of TRPM8 is completely abolished in the mutant ganglia. In addition, TRPM8 is not co-expressed with VR1, which marks a class of nociceptors that respond to capsaicin and noxious heat. This observation is confirmed by the lack of TRPM8 co-expression with either CGRP or IB4, two well-characterized antigenic markers found on nociceptive neurons (see Snider and McMahon, *Neuron*, 20:629-632 (1998); Tominaga et al., *Neuron*, 21:531-543 (1998)). This data strongly indicates that TRPM8 is expressed in a subpopulation of

thermoceptive/nociceptive neurons distinct from the well-characterized heat and pain sensing neurons marked by VR1, CGRP or IB4.

[0260] Following *in situ* hybridization, immunofluorescence is performed with anti-CGRP (1:100; Biogenesis), IB-4 (10 μ g/mL; Sigma), anti-VR1 (1/2000; Abcam), anti-NF150 (1/1000; Chemicon) and detected with FITC or CY3 (10 μ g/mL; Jackson Immunoresearch). Although all panels shown in these studies demonstrate lack of co-expression, this is not due to technical issues since additional probes/antibodies are used as controls to ensure our double-labeling protocol with the TRPM8 *in situ* probe is working.

EXAMPLE 9

10 Activation of TRPM8 Protein by Cold and Menthol

A. Effect of heat, capsaicin, cold and menthol upon intracellular calcium

[0261] Given the similarity of TRPM8 protein to TRPV family members and its unique expression pattern, the effects of heat, capsaicin, cold and menthol in mediating calcium influx are examined using transfected CHO-K1/FRT cells expressing TRPM8 protein and a fluorescent calcium imaging method as described in detail below.

[0262] To generate mouse TRPM8-expressing CHO cell lines, mouse TRPM8 cDNA are subcloned in pcDNA5 (Invitrogen), transfected into CHO-K1/FRT cells using Fugene 6 (Roche). The transfected cells are selected by growth in MEM medium containing 200 μ g/ μ L⁻¹ hygromycin (Gibco BRL). Populations are frozen at early passage numbers and these stocks are used for further studies. Stable clones that express the mRNAs are identified by Northern blot analysis as well as Southern blotting to confirm integration site (not shown). CHO cells do not express an endogenous TRPM8 isoform and therefore serve as a control along with a cell line stably transfected with a VR1-expressing plasmid.

[0263] Calcium imaging experiments are performed essentially as previously described (see Savidge et al., *Neuroscience*, 102:177-184 (2001)). Briefly, cells are plated on glass coverslips and loaded with Fura-2 acetoxymethyl ester (2.5-5 mM) and incubated for 30-60 minutes at room temperature in 1.5 mM of pluronic acid (Molecular Probes, Eugene, OR) in a HEPES-buffered saline (2 mM Ca²⁺). Coverslips are placed in a laminar flow perfusion chamber (Warner Instrument Corp.) and constantly perfused with HEPES-buffered saline (2 mM Ca²⁺) via a local perfusion pipette through which buffer and chilled

solutions are also applied. Chilled stimulations consist of a linear decrease ($\sim 1.1.5^{\circ}\text{C sec}^{-1}$) in perfusate temperature from 33°C to 10°C . Perfusate temperature is controlled by a regulated Peltier device and is monitored in the cell chamber by a miniature thermocouple. Alternatively, cells are plated on 24-well tissue culture plates, loaded with Fura-2 and application of solutions is performed with a 3 cc syringe over a period of 10 seconds. Images of Fura-2 loaded cells with the excitation wavelength alternating between 340 and 380 nm are captured with a cooled CCD camera. Following subtraction of background fluorescence, the ratio of fluorescence intensity at the two wavelengths is calculated. Ratio levels in groups of 20-40 individual cells are analyzed using MetaFluor (Universal Imaging Corporation). All graphs are averaged responses from groups of 20-30 individual cells from representative single experiments. All experiments are repeated on three separate occasions and similar results obtained. Hanks balanced salt solution (HBSS), phosphate buffered saline (PBS) and all cell culture reagents are obtained from Gibco BRL. Ruthenium red, capsaicin and menthol are obtained from Sigma.

[0264] The results of the above calcium imaging experiments are described below. Capsaicin ($10\text{ }\mu\text{M}$), an activator of VR1, does not evoke a response in TRPM8 expressing cells. Neither hypo-osmotic solutions, known to generate Ca^{2+} responses in TRPV3-expressing cells, or hypertonic buffer elicit a response in TRPM8 expressing cell lines (see Liedtke et al., *supra*, and Strohmann et al., *supra*). An increase in temperature ($25\text{-}50^{\circ}\text{C}$), a potent stimulus for VR1, also does not alter intracellular calcium levels. However, when the temperature is lowered from 25°C to 15°C , an increase in intracellular calcium is observed in TRPM8 expressing cells (Figures 7A and 8A). This response is not observed in non-transfected CHO cells or the VR1-expressing cell line (Figures 7A and 8A). Addition of a 10°C stimulus also evokes an influx of Ca^{2+} . This response is dependent on Ca^{2+} in the buffer, because removal of extracellular calcium suppresses the temperature response (Figures 7A and 8A). The dependence on outside calcium is indicative of a cation-permeable channel localized at the plasma membrane. A potent blocker of the heat response for VR1, ruthenium red (at $5\text{ }\mu\text{M}$), does not suppress the temperature response.

[0265] Since TRPM8 responds to a decrease in temperature, additional experiments are carried out to investigate the temperature threshold at which intracellular calcium ($[\text{Ca}^{2+}]_i$) begins to rise in TRPM8 expressing cells. Cells are incubated at 33°C (normal skin temperature) for several minutes followed by a decrease in temperature to

13°C . The temperature response in mouse TRPM8-CHO cells shows a threshold of $22\text{-}25^{\circ}\text{C}$ at which $[\text{Ca}^{2+}]_i$ starts to increase (Figure 7B), followed by a marked increase when the temperature of the buffer reached $\sim 15^{\circ}\text{C}$. These experiments indicate that at physiological relevant temperatures, the upper activation threshold for TRPM8 is $\sim 23^{\circ}\text{C}$ (Figure 7C).

[0266] Menthol, a compound commonly used for its cooling properties, is tested as a stimulus on TRPM8 expressing CHO cells. Non-transfected CHO cells are completely insensitive to menthol (tested up to 1 mM) (Figure 7D). However, upon treatment of TRPM8 cells (incubated at 23°C), intracellular fluorescence increases significantly within seconds in response to menthol concentrations of 10 and $100\text{ }\mu\text{M}$ (Figure 7D). Additionally, as with the temperature stimulus, depletion of calcium from the extracellular buffer suppresses the calcium response (Figure 7D). The effect that menthol has at different temperatures is also examined. Incubation of TRPM8 expressing cells at 33°C , reveals that $10\text{ }\mu\text{M}$ menthol does not induce a calcium response as observed at 23°C , but upon lowering the temperature to 30°C , intracellular calcium levels increases (Figure 7E). Menthol thus appears to mimic the effect of lowering the temperature on TRPM8 expressing cells.

B. Effect of cold and menthol upon conductance

[0267] To investigate the membrane responses to cold and menthol, voltage clamp experiments are carried out on TRPM8 expressing cells which are prepared as described above.

[0268] Cells are plated onto poly-D-lysine coated cover-slips for recording purposes and recordings undertaken 18-24 hours later. Experiments are carried out at room temperature using whole-cell voltage clamp technique, with an Axopatch 2B amplifier, filtered at 5 kHz and pClamp suite of software (Axon Instruments). Series resistant compensation is 80% for all experiments, using $2\text{-}5\text{ M}\Omega$ fire-polished pipettes. Recording solutions are as follows; pipette solution for all experiments is (mM) CsCl, 140 ; CaCl_2 , 1 ; EGTA, 10 ; HEPES, 10 ; MgATP, 2 ; titrated to pH 7.4 with CsOH. For menthol and cold activated currents the bath solution is (mM): NaCl, 140 ; KCl, 5 ; Glucose, 10 ; HEPES, 10 ; CaCl_2 , 2 ; MgCl_2 , 1 ; titrated to pH 7.4 with NaOH. Current-voltage relationships are used to evaluate reversal potentials with voltage ramps from -100 to $+60\text{ mV}$ (2 second duration). For the permeability studies for the monovalent ions the NaCl in a simplified bath solution (mM): NaCl, 140 ; Glucose, 10 ; HEPES, 10 ; CaCl_2 , 2 ; MgCl_2 , 1 , is substituted by either

equimolar CsCl or KCl (titrated with CsOH or KOH). For calcium permeability estimates, the bath solutions contains (mM) NaCl, 100; Glucose, 10 mM; Hepes, 10 mM (titrated with NaOH) plus 1 or 30 mM CaCl_2 . Osmolarity of solutions are adjusted by addition of sucrose. Permeability ratios for the monovalent cations to Na (P_X/P_{Na}) are calculated as follows:

$$P_X/P_{\text{Na}} = E_{\text{Na}} = \{RT/F\} \log (P_X/P_{\text{Na}})[X]_o/[Na]_o$$

where F is Faraday's constant, R is the universal gas constant and T is absolute temperature. For measurements of calcium permeability $P_{\text{Ca}}/P_{\text{Na}}$ is calculated as follows:

$$E_{\text{Na}} = \{RT/F\} \log ([Na]_o + 4B^+ [Ca]_o(2)) / ([Na]_o + 4B^+ [Ca]_o(1))$$

where $B^+ = P'_{\text{Ca}}/P_{\text{Na}}$ and $P'_{\text{Ca}} = P_{\text{Ca}}/(1 + e^{\frac{EF}{RT}})$ and $[Ca]_o(1)$ and $[Ca]_o(2)$ refer to the two different calcium concentrations. Local perfusion of menthol is via a TC³bip

temperature controller. A Marlow temperature controller is used for the cooling ramps.

[0269] The results of the voltage clamp studies carried out on TRPM8 expressing cells are described below. Temperature ramps from 35°C to 7-13°C evoke inward currents at a holding potential of -60 mV and outward currents at +40 or +60 mV. Currents increase in amplitude as the temperature is lowered and usually show some degree of desensitization at the coldest temperatures tested <10°C (Figure 9A). The temperature threshold for current activation shows no dependence on membrane potential and individual cells activated at temperatures between 19°C and 25°C, with a mean threshold of $21.79 \pm 0.64^\circ\text{C}$ ($n=5$). Analysis of the current-voltage relationships of the response to a cold stimulus with CsCl filled recording pipettes and a typical NaCl-based external solution reveals an outwardly rectifying current with a reversal potential (E_{rev}) close to 0 mV which is typical of a non-selective cation channel (Figure 9B).

[0270] Application of menthol evokes rapidly activating currents in TRPM8 expressing, but not in non-transfected CHO cells at temperatures above the threshold for cold activation (>23°C, Figure 10A). The menthol activated current shows pronounced outward rectification (Figure 10B) with an E_{rev} of -9.28 ± 0.75 mV ($n=12$) that is similar to the E_{rev} for the cold-activated current under the same ionic conditions. These currents could be inactivated by raising the temperature (see Figure 10A) suggesting that menthol shifts the threshold for activation to higher temperatures, which agrees with the calcium imaging experiments. To test this idea further, concentration-response curves for menthol-evoked currents at two temperatures (22°C and 35°C) are obtained using positive membrane potentials to increase the size of the currents (Figures 11A and 11B). The concentration-

response relationship is shifted to the left at the lower temperature with a marked increase in the maximum amplitudes (Figures 11A and 11B). Changes in E_{rev} are used to determine the ion selectivity of the menthol activated current. Isotonic replacement of the NaCl in the solution with KCl or CsCl, causes small positive shifts in E_{rev} , indicating that the TRPM8 channel discriminates poorly between these cations (data not shown). From the changes in E_{rev} measured on individual cells (external NaCl to KCl gives a shift of $+7.38 \pm 1.43$ mV, $n=7$; NaCl to CsCl gives a shift of $+9.09 \pm 0.36$ mV, $n=5$) a permeability sequence of $\text{Cs} > \text{K} > \text{Na}$ is calculated with $P_{\text{Ca}}/P_{\text{Na}} = 1.43$ and $P_{\text{K}}/P_{\text{Na}} = 1.34$. Relative calcium permeability is calculated from the E_{rev} values measured with different external calcium concentrations. Increasing the external calcium from 1-30 mM, in the absence of external Mg^{2+} ions, shifts E_{rev} by $+11.67 \pm 1.20$ mV, which corresponds to $P_{\text{Ca}}/P_{\text{Na}} = 0.97$. Thus TRPM8 is permeable to the monovalent cations, Na, K and Cs as well as the divalent cation calcium.

[0271] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

WE CLAIM:

1. An isolated TRPV3 nucleic acid molecule comprising a member selected from the group consisting of:
 - a) a polynucleotide that encodes a mouse TRPV3 protein comprising amino acid residues 1-791 of SEQ ID NO: 2;
 - b) a polynucleotide that encodes a mouse TRPV3 protein comprising amino acid residues 2-791 of SEQ ID NO: 2;
 - c) a polynucleotide that encodes a polypeptide that comprises one or more functional domains of a mouse TRPV3 protein;
 - d) a polynucleotide that encodes a human TRPV3 protein comprising amino acid residues 1-791 of SEQ ID NO: 5;
 - e) a polynucleotide that encodes a human TRPV3 protein comprising amino acid residues 2-791 of SEQ ID NO: 5;
 - f) a polynucleotide that encodes a polypeptide that comprises one or more functional domains of a human TRPV3 protein; and
 - g) a polynucleotide that is complementary to a polynucleotide of a) through f).
2. The TRPV3 nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a polydeoxyribonucleic acid (DNA).
3. The TRPV3 nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a polycyribonucleic acid (RNA).
4. The TRPV3 nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a) or b) and comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 3.
5. The TRPV3 nucleic acid molecule of claim 4, wherein the first polynucleotide is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 65-2440 of SEQ ID NO: 1.

6. The TRPV3 nucleic acid molecule of claim 4, wherein the first polynucleotide is 90% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 65-2440 of SEQ ID NO: 1.
7. The TRPV3 nucleic acid molecule of claim 4, wherein the first polynucleotide comprises a nucleotide sequence as set forth in nucleotides 65-2440 of SEQ ID NO: 1.
8. The TRPV3 nucleic acid molecule of claim 1, wherein the nucleic acid molecule is d) or e) and comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 6.
9. The TRPV3 nucleic acid molecule of claim 8, wherein the first polynucleotide is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 57-2432 of SEQ ID NO: 4.
10. The TRPV3 nucleic acid molecule of claim 9, wherein the first polynucleotide is 90% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 57-2432 of SEQ ID NO: 4.
11. The TRPV3 nucleic acid molecule of claim 9, wherein the first polynucleotide comprises a nucleotide sequence as set forth in nucleotides 57-2432 of SEQ ID NO: 4.
12. The TRPV3 nucleic acid molecule of claim 1, wherein the nucleic acid molecule is c) or f) and the polypeptide comprises one or more functional domains selected from the group consisting of:
 - a) an ankyrin domain;
 - b) a transmembrane region;
 - c) a pore loop region; and
 - d) a coiled-coil domain.
13. The TRPV3 nucleic acid molecule of claim 12, wherein the polypeptide comprises a pore loop region flanked by two transmembrane regions.

14. The TRPV3 nucleic acid molecule of claim 12, wherein the polypeptide comprises four ankyrin domains.

15. The TRPV3 nucleic acid molecule of claim 1, wherein the nucleic acid molecule further comprises a heterologous nucleic acid.

5 16. The TRPV3 nucleic acid molecule of claim 15, wherein the heterologous nucleic acid comprises a promoter operably linked to the TRPV3 polynucleotide.

17. The TRPV3 nucleic acid molecule of claim 15, wherein the heterologous nucleic acid comprises an expression vector.

10 18. A host cell that comprises a TRPV3 nucleic acid molecule of claim 15.

19. An isolated TRPV3 polypeptide comprising a member selected from the group consisting of:

a) a mouse TRPV3 protein comprising amino acid residues 1-791 of SEQ ID NO: 2;

15 b) a mouse TRPV3 protein comprising amino acid residues 2-791 of SEQ ID NO: 2;

c) one or more functional domains of a mouse TRPV3 protein;

d) a human TRPV3 protein comprising amino acid residues 1-791 of SEQ ID NO 5;

20 e) a human TRPV3 protein comprising amino acid residues 2-791 of SEQ ID NO 5; and

f) one or more functional domains of a human TRPV3 protein.

20 20. The TRPV3 polypeptide of claim 19, wherein the TRPV3 polypeptide is c) or f) and comprises one or more functional domains selected from the group consisting of:

a) an ankyrin domain;

b) a transmembrane region;

c) a pore loop region; and

d) a coiled-coil domain.

21. The TRPV3 polypeptide of claim 20, wherein the polypeptide comprises a pore loop region flanked by two transmembrane regions.

5 22. The TRPV3 polypeptide of claim 20, wherein the polypeptide comprises four ankyrin domains.

23. An antibody that specifically binds to a TRPV3 polypeptide of claim 19.

24. A method for identifying an agent that modulates TRPV3-mediated cation passage through a membrane, the method comprising:

10 a) providing a membrane that comprises a TRPV3 polypeptide of claim 19;

b) contacting the membrane with a candidate agent; and

15 c) determining whether passage of one or more cations through the membrane is increased in the presence of the candidate agent compared to passage in the absence of the candidate agent.

25. The method of claim 24, wherein the membrane comprises a cell and cation passage through the membrane is detected by measuring cation influx across the membrane into the cell.

20 26. The method of claim 25, wherein the cell comprises a promoter operably linked to a heterologous polynucleotide that encodes the TRPV3 polypeptide.

27. The method of claim 24, wherein cation passage through the membrane is detected by voltage clamping.

28. The method of claim 24, wherein cation passage through the membrane is detected by an ion sensitive dye or a membrane potential dye.

25 29. The method of claim 24, wherein the assay is conducted at a temperature of at least 33°C.

30. The method of claim 24, wherein the assay is conducted at a temperature of less than 52°C.

31. The method of claim 30, wherein the assay is conducted at a temperature of less than 43°C.

32. The method of claim 24, wherein the membrane is contacted with the candidate modulating agent in a well of a multiwell plate.

33. The method of claim 32, wherein the multiwell plate is a 96-, 384- or 1536-well plate.

34. The method of claim 24, wherein a candidate agent that reduces cation passage is further tested for ability to treat pain by administering the candidate agent to a test animal and determining whether the candidate agent decreases the test animal's response to a pain stimulus.

35. The method of claim 34, wherein the pain stimulus is exposure to a temperature above 33° C.

36. A method of reducing pain associated with TRPV3 activity, the method comprising administering to a subject suffering from pain an analgesically effective amount of a compound that reduces TRPV3-mediated cation passage through a membrane or reduces signal transduction from a TRPV3 polypeptide to a DRG neuron.

37. The method of claim 36, wherein the pain is associated with one or more of heat exposure, inflammation, or tissue damage.

38. The method of claim 36, wherein the compound is selected from the group consisting of:

- a) an antibody that specifically binds to a TRPV3 polypeptide;
- b) an antisense polynucleotide, ribozyme, or an interfering RNA that reduces expression of a TRPV3 polypeptide; and
- c) a chemical compound that reduces cation passage through a membrane that comprises a TRPV3 polypeptide.

39. The method of claim 38, wherein the chemical compound has a molecular weight of 1000 daltons or less.

40. A method for determining whether pain in a subject is mediated by TRPV3, the method comprising:

- a) obtaining a sample from a region of the subject at which the pain is felt; and
- b) testing the sample to determine whether a TRPV3 polypeptide or TRPV3 polynucleotide is present in the sample.

41. The method of claim 40, wherein the presence of a TRPV3 polypeptide in the sample is detected by determining whether cation passage across membranes of cells in the sample is mediated by a TRPV3 polypeptide.

42. The method of claim 41, wherein TRPV3 involvement in mediating cation passage across membranes of the cells is determined by detecting an increase in cation passage across membranes of the cells when assayed above 33°C compared to cation passage when assayed below 33°C.

43. The method of claim 40, wherein the presence of a TRPV3 polypeptide in the sample is detected by contacting the sample with a reagent that specifically binds to a TRPV3 polypeptide.

44. The method of claim 43, wherein the reagent comprises an antibody.

45. The method of claim 40, wherein the presence of a TRPV3 polynucleotide in the sample is detected by contacting nucleic acids from the sample with a test polynucleotide that can hybridize to a TRPV3 polynucleotide.

46. The method of claim 45, wherein the test polynucleotide comprises an oligonucleotide at least 10 nucleotides in length.

47. The method of claim 45, wherein the method comprises amplification of a TRPV3 polynucleotide, if present in the sample.

48. The method of claim 47, wherein the amplification comprises polymerase chain reaction or ligase chain reaction.
49. The method of claim 45, wherein the test polynucleotide is attached to a solid support.
50. The method of claim 49, wherein the solid support comprises a microchip.
51. An isolated TRPV4 nucleic acid molecule comprising a member selected from the group consisting of:
- a polynucleotide that encodes a mouse TRPV4 protein comprising amino acid residues 1-871 of SEQ ID NO: 14;
 - a polynucleotide that encodes a mouse TRPV4 protein comprising amino acid residues 2-871 of SEQ ID NO: 14;
 - a polynucleotide that encodes a polypeptide that comprises one or more functional domains of a mouse TRPV4 protein;
 - a polynucleotide that encodes a human TRPV4 protein comprising amino acid residues 1-871 of SEQ ID NO 17;
 - a polynucleotide that encodes a human TRPV4 protein comprising amino acid residues 2-871 of SEQ ID NO 17;
 - a polynucleotide that encodes a polypeptide that comprises one or more functional domains of a human TRPV4 protein; and
 - a polynucleotide that is complementary to a polynucleotide of a) through f).
52. The TRPV4 nucleic acid molecule of claim 51, wherein the nucleic acid molecule is a polydeoxyribonucleic acid (DNA).
53. The TRPV4 nucleic acid molecule of claim 51, wherein the nucleic acid molecule is a polyribonucleic acid (RNA).

54. The TRPV4 nucleic acid molecule of claim 51, wherein the nucleic acid molecule is a) or b) and comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 15.

55. The TRPV4 nucleic acid molecule of claim 54, wherein the first polynucleotide is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 156-2771 of SEQ ID NO: 13.

56. The TRPV4 nucleic acid molecule of claim 54, wherein the first polynucleotide is 90% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 156-2771 of SEQ ID NO: 13.

57. The TRPV4 nucleic acid molecule of claim 56, wherein the first polynucleotide comprises a nucleotide sequence as set forth in nucleotides 156-2771 of SEQ ID NO: 13.

58. The TRPV4 nucleic acid molecule of claim 51, wherein the nucleic acid molecule is d) or e) and comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 18.

59. The TRPV4 nucleic acid molecule of claim 58, wherein the first polynucleotide is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 16.

60. The TRPV4 nucleic acid molecule of claim 58, wherein the first polynucleotide is 90% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 16.

61. The TRPV4 nucleic acid molecule of claim 60, wherein the first polynucleotide comprises a nucleotide sequence as set forth in SEQ ID NO: 16.

62. The TRPV4 nucleic acid molecule of claim 51, wherein the nucleic acid molecule is c) or f) and the polypeptide comprises one or more functional domains selected from the group consisting of:

- an ankyrin domain;

- b) a transmembrane region;
 c) a pore loop region; and
 d) a coiled-coil domain.
63. The TRPV4 nucleic acid molecule of claim 62, wherein the polypeptide comprises a pore loop region flanked by two transmembrane regions.
64. The TRPV4 nucleic acid molecule of claim 62, wherein the polypeptide comprises three ankyrin domains.
65. The TRPV4 nucleic acid molecule of claim 51, wherein the nucleic acid molecule further comprises a heterologous nucleic acid.
66. The TRPV4 nucleic acid molecule of claim 65, wherein the heterologous nucleic acid comprises a promoter operably linked to the TRPV4 polynucleotide.
67. The TRPV4 nucleic acid molecule of claim 65, wherein the heterologous nucleic acid comprises an expression vector.
68. A host cell that comprises a TRPV4 nucleic acid molecule of claim 65.
69. An isolated TRPV4 polypeptide comprising a member selected from the group consisting of:
 a) a mouse TRPV4 protein comprising amino acid residues 1-871 of SEQ ID NO: 14;
 b) a mouse TRPV4 protein comprising amino acid residues 2-871 of SEQ ID NO: 14;
 c) one or more functional domains of a mouse TRPV4 protein;
 d) a human TRPV4 protein comprising amino acid residues 1-871 of SEQ ID NO 17;
 e) a human TRPV4 protein comprising amino acid residues 2-871 of SEQ ID NO 17; and
 f) one or more functional domains of a human TRPV4 protein.

70. The TRPV4 polypeptide of claim 69, wherein the polypeptide is c) or f) and comprises one or more functional domains selected from the group consisting of:
 a) an ankyrin domain;
 b) a transmembrane region;
 c) a pore loop region; and
 d) a coiled-coil domain.
71. The TRPV4 polypeptide of claim 70, wherein the polypeptide comprises a pore loop region flanked by two transmembrane regions.
72. The TRPV4 polypeptide of claim 70, wherein the polypeptide comprises three ankyrin domains.
73. An antibody that specifically binds to a TRPV4 polypeptide of claim 69.
74. A method for identifying an agent that modulates TRPV4-mediated cation passage through a membrane, the method comprising:
 a) providing a membrane that comprises a TRPV4 polypeptide of claim 69;
 b) contacting the membrane with a candidate agent; and
 c) determining whether passage of one or more cations through the membrane is increased in the presence of the candidate agent compared to passage in the absence of the candidate agent.
75. The method of claim 74, wherein the membrane comprises a cell and cation passage through the membrane is detected by measuring cation influx across the membrane into the cell.
76. The method of claim 75, wherein the cell comprises a promoter operably linked to a heterologous polynucleotide that encodes the TRPV4 polypeptide.
77. The method of claim 74, wherein cation passage through the membrane is detected by voltage clamping.

78. The method of claim 74, wherein cation passage through the membrane is detected by an ion sensitive dye or a membrane potential dye.

79. The method of claim 74, wherein the membrane is contacted with the candidate modulating agent in a well of a multiwell plate.

5 80. The method of claim 79, wherein the multiwell plate is a 96-, 384- or 1536-well plate.

81. The method of claim 74, wherein a candidate agent that reduces cation passage is further tested for ability to treat pain by administering the candidate agent to a test animal and determining whether the candidate agent decreases the test animal's response to a pain stimulus.

82. The method of claim 81, wherein the pain is neuropathic pain.

83. A method of reducing pain associated with TRPV4 activity, the method comprising administering to a subject suffering from pain an analgesically effective amount of a compound that reduces TRPV4-mediated cation passage through a membrane or reduces signal transduction from a TRPV4 polypeptide to a DRG neuron.

84. The method of claim 83, wherein the pain is neuropathic pain.

85. The method of claim 83, wherein the compound is selected from the group consisting of:

- a) an antibody that specifically binds to a TRPV4 polypeptide;
- b) an antisense polynucleotide, ribozyme, or an interfering RNA that reduces expression of a TRPV4 polypeptide; and
- c) a chemical compound that reduces cation passage through a membrane that comprises a TRPV4 polypeptide.

86. The method of claim 85, wherein the chemical compound has a molecular weight of 1000 daltons or less.

87. A method for determining whether pain in a subject is mediated by TRPV4, the method comprising:

- a) obtaining a sample from a region of the subject at which the pain is felt; and
- b) testing the sample to determine whether a TRPV4 polypeptide or TRPV4 polynucleotide is present in the sample.

5 88. The method of claim 87, wherein the presence of a TRPV4 polypeptide in the sample is detected by determining whether cation passage across membranes of cells in the sample is mediated by a TRPV4 polypeptide.

89. The method of claim 87, wherein the presence of a TRPV4 polypeptide in the sample is detected by contacting the sample with a reagent that specifically binds to a TRPV4 polypeptide.

90. The method of claim 89, wherein the reagent comprises an antibody.

91. The method of claim 87, wherein the presence of a TRPV4 polynucleotide in the sample is detected by contacting nucleic acids from the sample with a test polynucleotide that can hybridize to a TRPV4 polynucleotide.

92. The method of claim 91, wherein the test polynucleotide comprises an oligonucleotide at least 10 nucleotides in length.

93. The method of claim 91, wherein the method comprises amplification of a TRPV4 polynucleotide, if present in the sample.

94. The method of claim 93, wherein the amplification comprises polymerase chain reaction or ligase chain reaction.

95. The method of claim 91, wherein the test polynucleotide is attached to a solid support.

96. The method of claim 95, wherein the solid support comprises a microchip.

97. An isolated TRPM8 nucleic acid molecule comprising a member selected from the group consisting of:

- a) a polynucleotide that encodes a mouse TRPM8 protein comprising amino acid residues 1-1104 of SEQ ID NO: 8;
- b) a polynucleotide that encodes a mouse TRPM8 protein comprising amino acid residues 2-1104 of SEQ ID NO: 8;
- c) a polynucleotide that encodes a polypeptide that comprises one or more functional domains of a mouse TRPM8 protein;
- d) a polynucleotide that encodes a human TRPM8 protein comprising amino acid residues 1-1268 of SEQ ID NO 11;
- e) a polynucleotide that encodes a human TRPM8 protein comprising amino acid residues 2-1268 of SEQ ID NO 11;
- f) a polynucleotide that encodes a polypeptide that comprises one or more functional domains of a human TRPM8 protein; and
- g) a polynucleotide that is complementary to a polynucleotide of a) through f).

15 98. The TRPM8 nucleic acid molecule of claim 97, wherein the nucleic acid molecule is a polydeoxyribonucleic acid (DNA).

99. The TRPM8 nucleic acid molecule of claim 97, wherein the nucleic acid molecule is a polynucleic acid (RNA).

100. The TRPM8 nucleic acid molecule of claim 97, wherein the nucleic acid molecule is a) or b) and comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 9.

101. The TRPM8 nucleic acid molecule of claim 100, wherein the first polynucleotide is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 448-3762 of SEQ ID NO: 7.

25 102. The TRPM8 nucleic acid molecule of claim 100, wherein the first polynucleotide is 90% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 448-3762 of SEQ ID NO: 7.

103. The TRPM8 nucleic acid molecule of claim 102, wherein the first polynucleotide comprises a nucleotide sequence as set forth in nucleotides 448-3762 of SEQ ID NO: 7.

104. The TRPM8 nucleic acid molecule of claim 97, wherein the nucleic acid molecule is d) or e) and comprises a first polynucleotide 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in SEQ ID NO: 12.

105. The TRPM8 nucleic acid molecule of claim 104, wherein the first polynucleotide is 80% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 61-4821 of SEQ ID NO: 10.

106. The TRPM8 nucleic acid molecule of claim 104, wherein the first polynucleotide is 90% or more identical to a second polynucleotide having a nucleotide sequence as set forth in nucleotides 61-4821 of SEQ ID NO: 10.

107. The TRPM8 nucleic acid molecule of claim 106, wherein the first polynucleotide comprises a nucleotide sequence as set forth in nucleotides 61-4821 of SEQ ID NO: 10.

108. The TRPM8 nucleic acid molecule of claim 97, wherein the nucleic acid molecule is c) or f) and the polypeptide comprises one or more functional domains selected from the group consisting of:

- a) a transmembrane region;
- b) a pore loop region; and
- c) a coiled-coil domain.

109. The TRPM8 nucleic acid molecule of claim 108, wherein the polypeptide comprises a pore loop region flanked by two transmembrane regions.

25 110. The TRPM8 nucleic acid molecule of claim 97, wherein the nucleic acid molecule further comprises a heterologous nucleic acid.

111. The TRPM8 nucleic acid molecule of claim 110, wherein the heterologous nucleic acid comprises a promoter operably linked to the TRPM8 polynucleotide.
112. The TRPM8 nucleic acid molecule of claim 110, wherein the heterologous nucleic acid comprises an expression vector.
113. A host cell that comprises a TRPM8 nucleic acid molecule of claim 97.
114. An isolated TRPM8 polypeptide comprising a member selected from the group consisting of:
- a mouse TRPM8 protein comprising amino acid residues 1-1104 of SEQ ID NO: 8;
 - a mouse TRPM8 protein comprising amino acid residues 2-1104 of SEQ ID NO: 8;
 - one or more functional domains of a mouse TRPM8 protein;
 - a human TRPM8 protein comprising amino acid residues 1-1268 of SEQ ID NO 11;
 - a human TRPM8 protein comprising amino acid residues 2-1268 of SEQ ID NO 11; and
 - one or more functional domains of a human TRPM8 protein.
115. The TRPM8 polypeptide of claim 114, wherein the nucleic acid molecule is c) or f) and the functional domains comprise one or more members selected from the group consisting of:
- a transmembrane region;
 - a pore loop region; and
 - a coiled-coil domain.
116. The TRPM8 polypeptide of claim 115, wherein the polypeptide comprises a pore loop region flanked by two transmembrane regions.
117. An antibody that specifically binds to a TRPM8 polypeptide of claim 114.

118. A method for identifying an agent that modulates TRPM8-mediated cation passage through a membrane, the method comprising:
- providing a membrane that comprises a TRPM8 polypeptide of claim 114;
 - contacting the membrane with a candidate agent; and
 - determining whether passage of one or more cations through the membrane is increased in the presence of the candidate agent compared to passage in the absence of the candidate agent.
119. The method of claim 118, wherein the membrane comprises a cell and cation passage through the membrane is detected by measuring cation influx across the membrane into the cell.
120. The method of claim 119, wherein the cell comprises a promoter operably linked to a heterologous polynucleotide that encodes the TRPM8 polypeptide.
121. The method of claim 118, wherein cation passage through the membrane is detected by voltage clamping.
122. The method of claim 118, wherein cation passage through the membrane is detected by an ion sensitive dye or a membrane potential dye.
123. The method of claim 118, wherein the membrane is contacted with the candidate modulating agent in a well of a multiwell plate.
124. The method of claim 123, wherein the multiwell plate is a 96-, 384- or 1536-well plate.
125. The method of claim 118, wherein the assay is to identify antagonists of TRPM8-mediated cation passage and is conducted at a temperature of less than 20°C and/or in the presence of menthol.
126. The method of claim 125, wherein a candidate agent that reduces cation passage is further tested for ability to treat pain by administering the candidate agent to a test

animal and determining whether the candidate agent decreases the test animal's response to a pain stimulus.

127. The method of claim 126, wherein the pain stimulus is cold.

128. The method of claim 118, wherein the assay is to identify agonists of TRPM8-mediated cation passage and is conducted at a temperature of greater than 20°C.

129. The method of claim 128, wherein an agonist of TRPM8-mediated cation passage is used as a fragrance or a flavor enhancer.

130. A method of reducing pain associated with TRPM8 activity, the method comprising administering to a subject suffering from pain an analgesically effective amount of a compound that reduces TRPM8-mediated cation passage through a membrane or reduces signal transduction from a TRPM8 polypeptide to a DRG neuron.

131. The method of claim 130, wherein the pain is associated with one or more of cold exposure, inflammation, or tissue damage.

132. The method of claim 130, wherein the compound is selected from the group consisting of:

- a) an antibody that specifically binds to a TRPM8 polypeptide;
- b) an antisense polynucleotide, ribozyme, or an interfering RNA that reduces expression of a TRPM8 polypeptide; and
- c) a chemical compound that reduces cation passage through a membrane that comprises a TRPM8 polypeptide.

133. The method of claim 132, wherein the chemical compound has a molecular weight of 1000 daltons or less.

134. A method for determining whether pain in a subject is mediated by TRPM8, the method comprising:

- a) obtaining a sample from a region of the subject at which the pain is felt; and

- b) testing the sample to determine whether a TRPM8 polypeptide or TRPM8 polynucleotide is present in the sample.

135. The method of claim 134, wherein the presence of a TRPM8 polypeptide in the sample is detected by determining whether cation passage across membranes of cells in the sample is mediated by a TRPM8 polypeptide.

136. The method of claim 135, wherein TRPM8 involvement in mediating cation passage across membranes of the cells is determined by detecting an increase or decrease in cation passage across membranes of the cells when assayed below 20°C and/or in the presence of menthol, compared to cation passage when assayed above 20°C and/or in the absence of menthol.

137. The method of claim 134, wherein the presence of a TRPM8 polypeptide in the sample is detected by contacting the sample with a reagent that specifically binds to a TRPM8 polypeptide.

138. The method of claim 137, wherein the reagent comprises an antibody.

139. The method of claim 134, wherein the presence of a TRPM8 polynucleotide in the sample is detected by contacting nucleic acids from the sample with a test polynucleotide that can hybridize to a TRPM8 polynucleotide.

140. The method of claim 139, wherein the test polynucleotide comprises an oligonucleotide at least 10 nucleotides in length.

141. The method of claim 139, wherein the method comprises amplification of a TRPM8 polynucleotide, if present in the sample.

142. The method of claim 141, wherein the amplification comprises polymerase chain reaction or ligase chain reaction.

143. The method of claim 139, wherein the test polynucleotide is attached to a solid support.

144. The method of claim 143, wherein the solid support comprises a microchip.
145. A method for identifying an agent useful in the modulation of a mammalian sensory response, the method comprising:
- a) contacting a candidate agent with a test system that comprises a receptor polypeptide selected from the group consisting of TRPM8, TRPV3 and TRPV4; and
 - b) detecting a change in activity of the receptor polypeptide in the presence of the candidate agent as compared to the activity of the receptor polypeptide in the absence of the agent, thereby identifying an agent that modulates receptor activity.
146. The method of claim 145, wherein the sensory response is response to cold and the polypeptide is a TRPM8 polypeptide.
147. The method of claim 146, wherein the TRPM8 polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 8 or SEQ ID NO: 11.
148. The method of claim 145, wherein the sensory response is response to warm or hot temperatures and the polypeptide is a TRPV3 polypeptide.
149. The method of claim 148, wherein the TRPV3 polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 5.
150. The method of claim 145, wherein the sensory response neuropathic pain and the polypeptide is a TRPV4 polypeptide.
151. The method of claim 150, wherein the TRPV4 polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 14 or SEQ ID NO: 17.
152. The method of claim 145, wherein the method further comprises administering the agent that modulates receptor activity to a test subject, and thereafter detecting a change in the sensory response in the test subject.

153. The method of claim 145, wherein the test system comprises a membrane that comprises the receptor polypeptide.
154. The method of claim 153, wherein the test system comprises a cell that expresses a heterologous polynucleotide that encodes the receptor polypeptide.
155. The method of claim 154, wherein the cell is substantially isolated and the contacting is performed *in vitro*.
156. The method of claim 154, wherein the cell is present in an organism and the contacting is performed *in vivo*.
157. The method of claim 145, wherein the receptor activity comprises increased or decreased Ca^{2+} passage through the membrane that comprises the receptor polypeptide.
158. The method of claim 157, wherein the membrane comprises a substantially purified cell membrane.
159. The method of claim 157, wherein the membrane comprises a liposome.
160. A method for monitoring the efficacy of a treatment of a subject suffering from pain, the method comprising:
- a) obtaining, at two or more time points in the course of treatment for pain, a sample from a region of the subject at which the pain is felt; and
 - b) testing the samples to determine whether a reduction is observed from one time point to another in amount or activity of one or more members selected from the group consisting of: a TRPV3 polypeptide, a TRPV3 mRNA, a TRPV4 polypeptide, a TRPV4 mRNA, a TRPM8 polypeptide, and a TRPM8 mRNA.
161. The method of claim 160, wherein one of the time points is prior to administration of the treatment for pain.

162. An assay capable of detecting the expression of one or more of TRPV3, TRPV4 or TRPM8 in human tissue, the assay selected from the group consisting of:

- a) an assay comprising contacting a human tissue sample with monoclonal antibodies binding to TRPV3, TRPV4 or TRPM8 and determining whether the monoclonal antibodies bind to polypeptides in the sample; and
- b) an assay comprising contacting a human tissue sample with an oligonucleotide that is capable of hybridizing to a nucleic acid that encodes TRPV3, TRPV4 or TRPM8.

163. The assay of claim 162, wherein the assay comprises contacting a human tissue sample with a pair of oligonucleotides that are capable of hybridizing to a nucleic acid that encodes TRPV3, TRPV4 or TRPM8 and subjecting the sample to polymerase chain reaction.

164. The assay of claim 162, wherein the assay comprises contacting a human tissue sample with an oligonucleotide array that comprises one or more oligonucleotides that are capable of hybridizing to a nucleic acid that encodes TRPV3, TRPV4 or TRPM8.

165. The assay of claim 162, wherein the human tissue sample is obtained from a site of pain.

166. A method of treating pain, the method comprising identifying a patient suffering from pain mediated by one or more polypeptides selected from the group consisting of TRPV3, TRPV4 and TRPM8 by measuring expression of the polypeptide in tissue from such patient, and administering to such patient an analgesically effective amount of an agent which inhibits the polypeptide.

167. A method for identifying an agent useful in the treatment of pain, the method comprising:

- a) administering a candidate agent to a mammal suffering from pain;
- b) in a sample obtained from the mammal, detecting an activity or amount of one or more members selected from the group consisting

of: a TRPV3 polypeptide, a TRPV3 mRNA, a TRPV4 polypeptide, a TRPV4 mRNA, a TRPM8 polypeptide, and a TRPM8 mRNA; and comparing the amount or activity of the member in the presence of the candidate agent with the amount or activity of the member in a sample obtained from the mammal in the absence of the candidate agent, wherein a decrease in amount or activity of the member in the sample in the presence of the candidate agent relative to the amount or activity in the absence of the candidate agent is indicative of an agent useful in the treatment of pain.

168. A method of identifying an agent that binds to and/or modulates the activity of an mRNA or polypeptide encoded by a TRPV3, TRPV4, or TRPM8 nucleic acid, the method comprising:

- a) contacting an isolated cell which expresses a heterologous TRPV3, TRPV4, or TRPM8 nucleic acid encoding a polypeptide with the agent; and
- b) determining binding and/or modulation of the activity of the mRNA or polypeptide by the agent, to identify agents which bind with and/or modulate the activity of the polypeptide.

Figure 2D

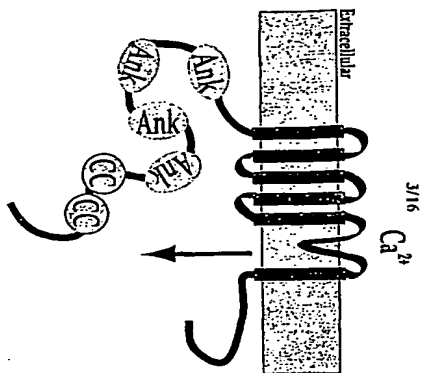


Figure 2E

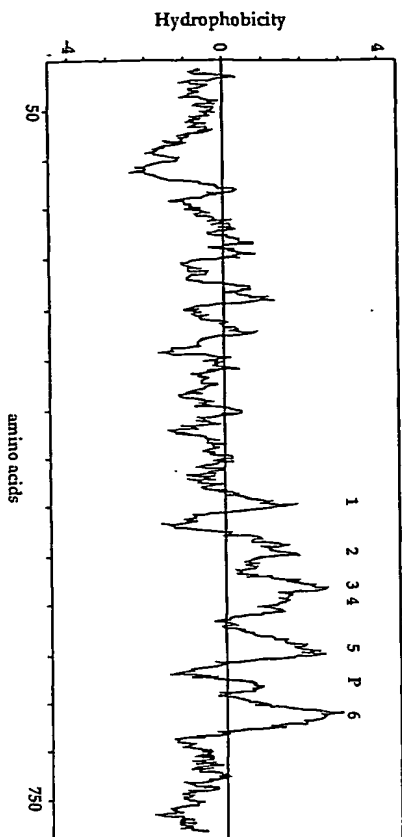


Figure 2F

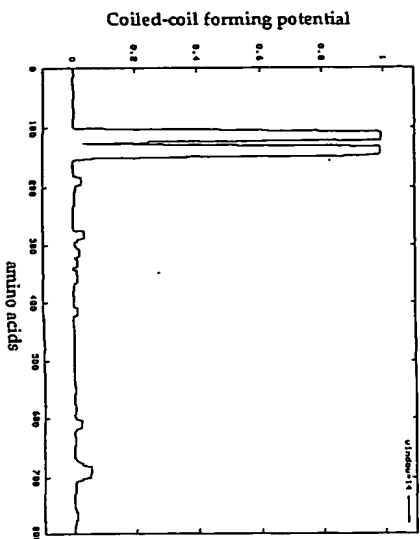


Figure 3

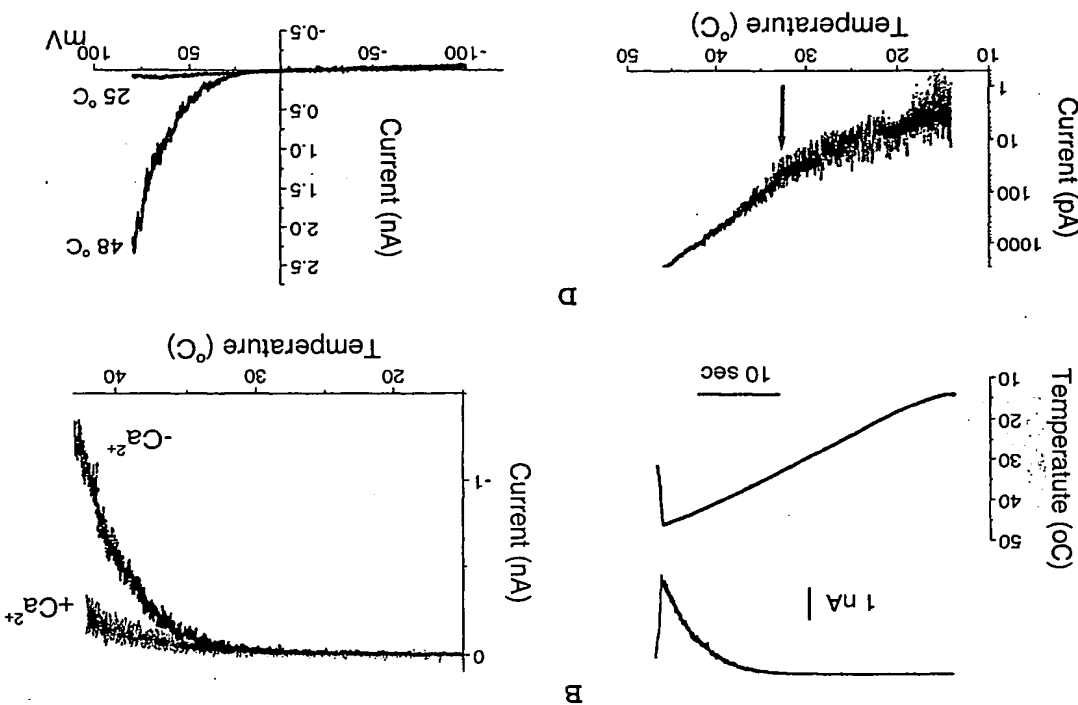


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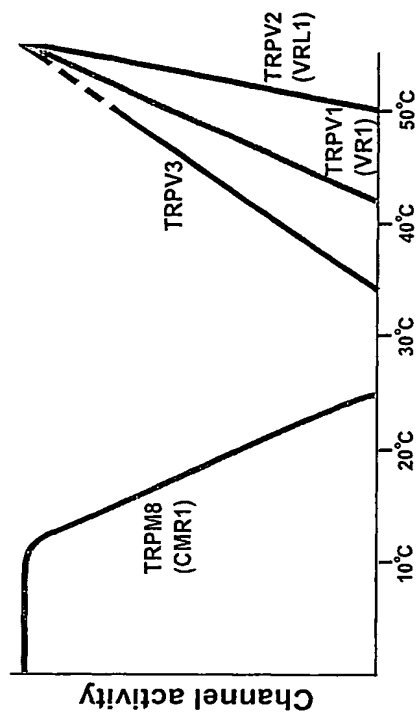
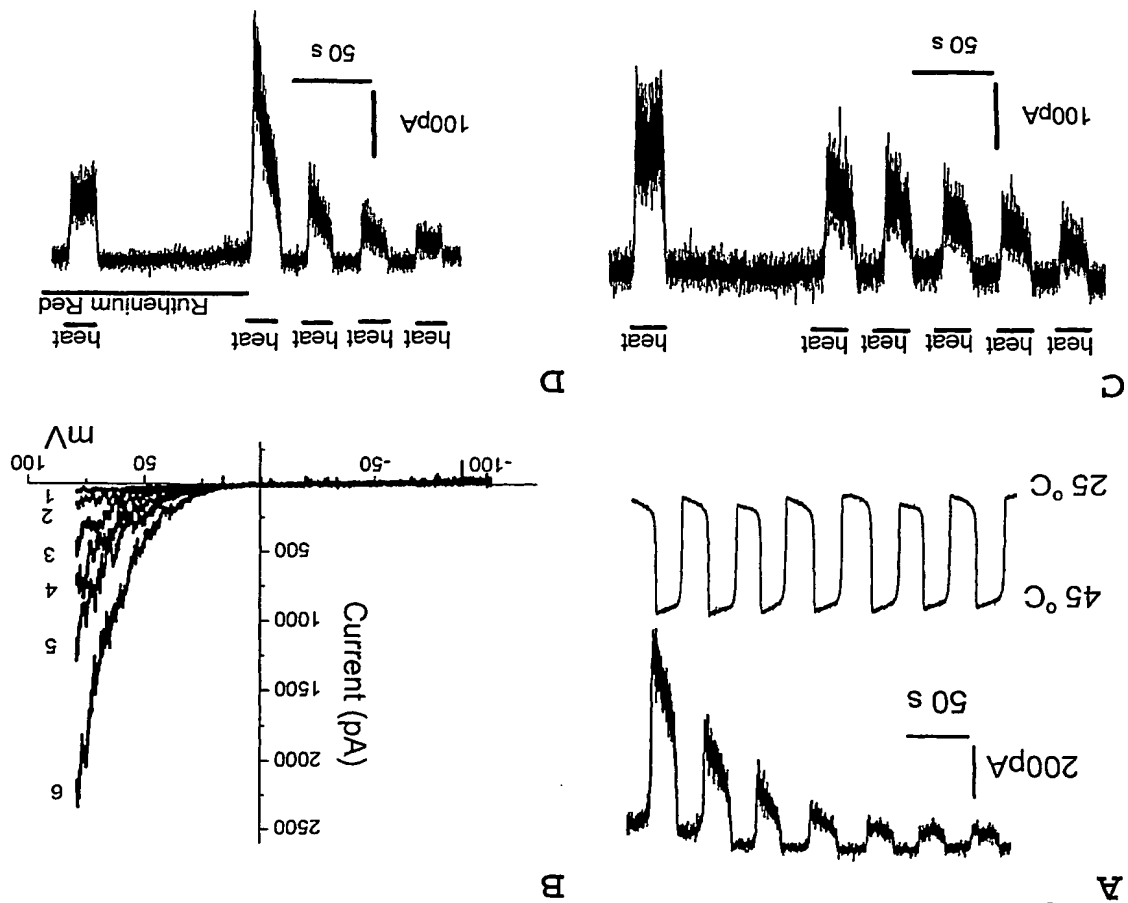


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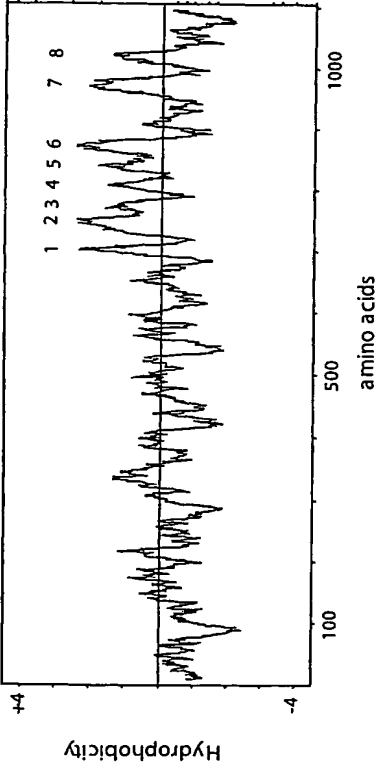


Figure 6D

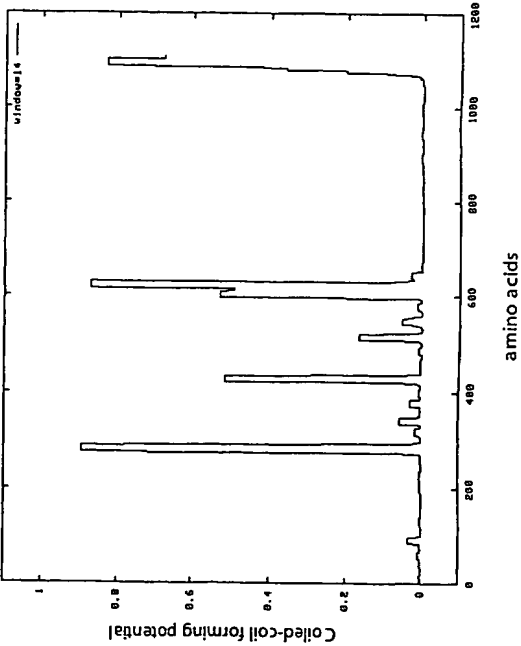


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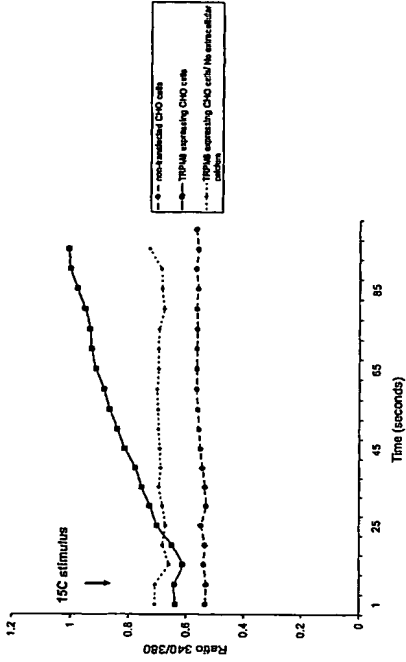


Figure 7B

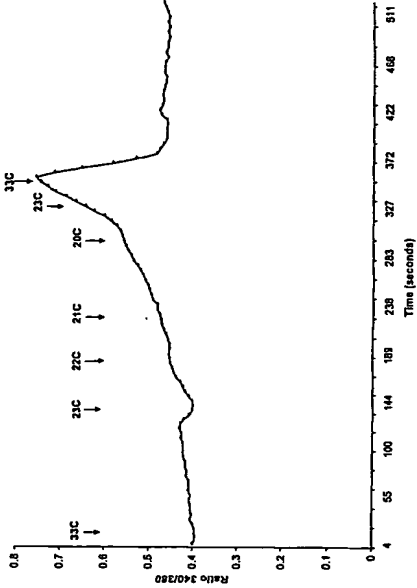


Figure 8

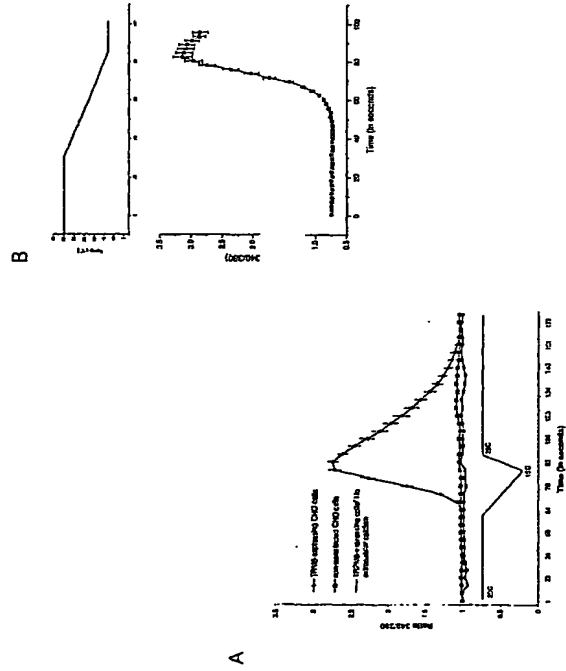


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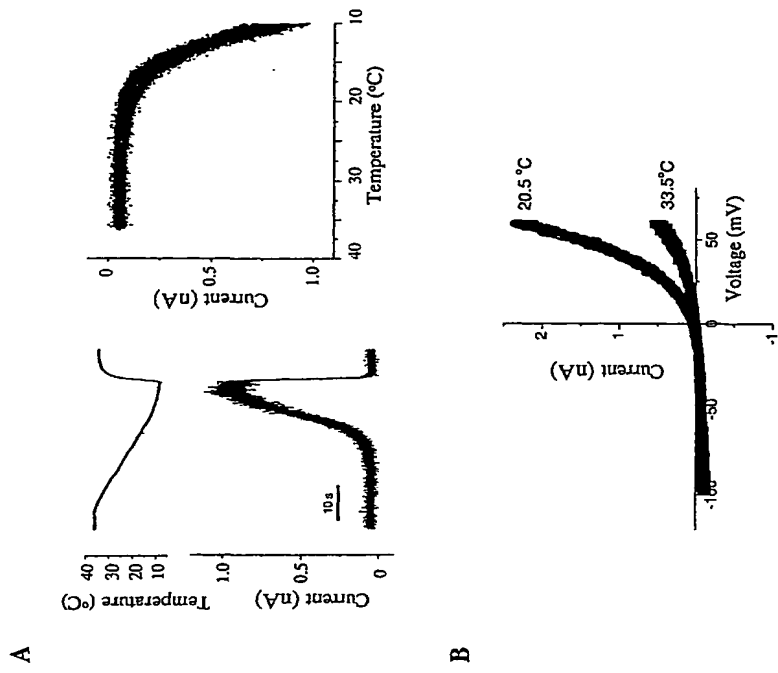


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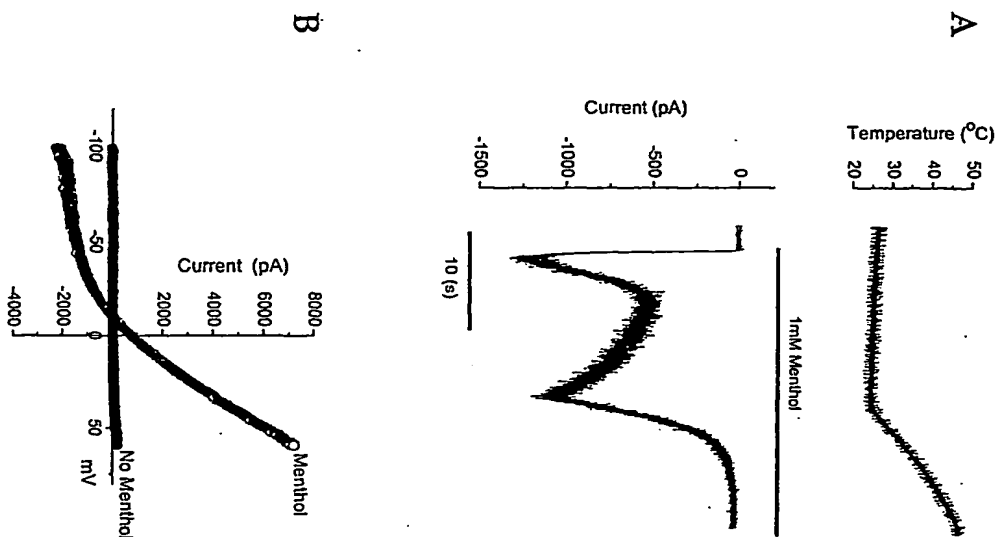
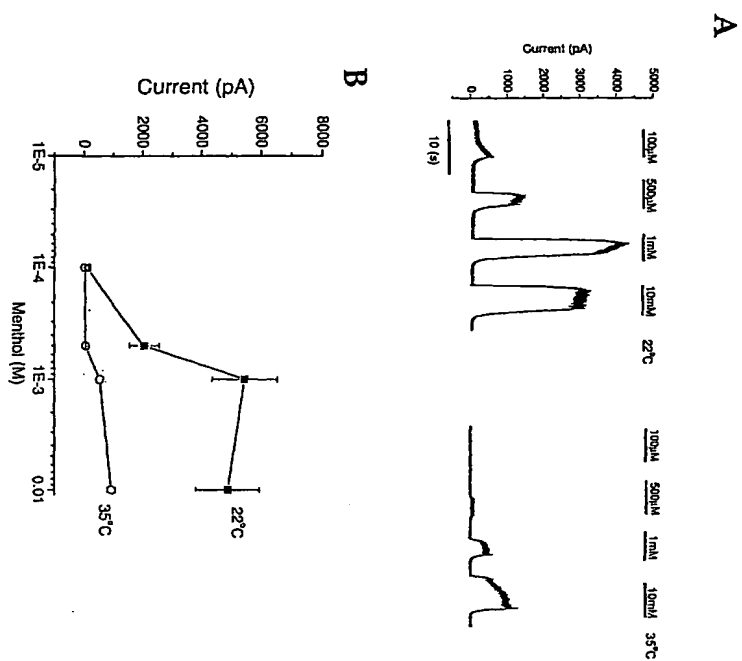


Figure 11



SEQUENCE LISTING

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Andrea Peier
Peter McIntyre
Stuart Bevan
Chuanzheng Song
Pamposh Ganju

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gar ggn tgy gtn gar gar ytn mgn gar ytn car gar ytn car gar Glu Gly Cys Val Gln Leu Arg Gln Leu Leu Gln Asp Leu Gln Asp	130	135	140	432	aay wsn gtn ytn gar ath ath gtn tay aay acn aay ath gar aay mgn Aon Ser Val Leu Gln Ile Ile Val Tyr Aon Thr Aon Ile Asp Aon Arg	405	410	1248
ytn tgy mgn mgn mgn ggn ytn gar ytn ccn gay tly ytn atg cay Leu Cys Arg Arg Arg Arg Gly Leu Asp Val Pro Asp Phe Leu Met His	145	150	155	480	cay gar atg ytn acn ytn gar ccn ytn cay acn ytn ytn cay acn aar His Gln Met Leu Thr Leu Gln Pro His Thr His Thr Leu His Thr lys	420	425	1296
aar ytn acn gcn wsn gay acn ggn aar acn tgy ytn atg aar gcn ytn lys Leu Thr Ala Ser Asp Thr Gly lys Thr Cys Leu Met lys Ala Leu	165	170	175	528	tgg aar aar tly gcn aar tay atg tly tly ytn wsn tly tgy tly tay Trp lys lys Phe Ala lys Tyr Met Phe Phe Leu Ser Cys Phe Tyr	435	440	1344
ytn aay ath aay ccn aay acn aar gar ath gtn mgn ath ytn ytn gcn Leu Aon Ile Aon Pro Aon Thr lys Ile Val Arg Ile Leu Leu Ala	180	185	190	576	tly tly tay aay ath acn ytn acn ytn gtn wsn tay tay mgn ccn mgn Phe Phe Tyr Aon Ile Thr Leu Thr Leu Val Ser Tyr Tyr Arg Pro Arg	450	455	1392
tly gcn gar gar aay gay ath ytn gay mgn tly ath aay gcn gar tay Phe Ala Gln Gln Aon Asp Ile Leu Asp Arg Phe Ile Aon Ala Gln Tyr	195	200	205	624	gar gar gar gar ytn ccn cay ccn ytn gtn ytn acn cay aar atg wsn Gln Asp Gln Asp Leu Pro His Pro Leu Ala Leu Thr His lys Met Ser	465	470	1440
acn gar gar gcn tay gar ggn car acn gcn ytn aay ath gcn ath gar Thr Gln Gln Ala Tyr Gln Gly Gln Thr Ala Leu Aon Ile Ala Ile Gln	210	215	220	672	tgg ytn car ytn ytn ggn mgn atg tly gtn ytn ath tgg gcn acn tgy Trp Leu Gln Leu Leu Gly Arg Met Phe Val Leu Ile Trp Ala Thr Cys	485	490	1488
mgn mgn car ggn gay ath acn gcn gtn ytn ath gcn gcn ggn gcn gay Arg Arg Gln Gly Asp Ile Thr Ala Val Leu Ile Ala Ala Gly Ala Asp	225	230	235	720	ath wsn gtn aar gar ggn ath gcn ath tly ytn ytn mgn ccn wsn gay Ile Ser Val lys Gln Gly Ile Ala Ile Phe Leu Leu Arg Pro Ser Asp	500	505	1536
gtn aay gcn cay gcn aar ggn gtn tly tly aay ccn aar tay car cay Val Aon Ala His Ala lys Gly Val Phe Aon Pro lys Tyr Tyr His	245	250	255	768	ytn car wsn ath ytn wsn gay gcn tgg tly cay tly gtn tly tly gtn Leu Gln Ser Ile Leu Ser Asp Ala Trp Phe His Phe Val Phe Val	515	520	1584
gar ggn tly tay tly ggn gar acn ccn ytn gcn ytn gcn gcn tgy acn Gln Gly Phe Tyr Phe Gly Gln Thr Pro Leu Ala Leu Ala Ala Cys Thr	260	265	270	816	car gcn gtn ytn gtn ath ytn wsn gtn tly ytn tay ytn tly gcn tay Gln Ala Val Leu Val Ile Leu Ser Val Phe Leu Tyr Leu Phe Ala Tyr	530	535	1632
aay car ccn gar ath gtn car ytn ytn atg gar aay gar car acn gay Aon Gln Pro Gln Ile Val Gln Leu Leu Met Gln Aon Gln Thr Asp	275	280	285	864	aar gar tay ytn gcn tgy ytn gtn ytn gcn atg gcn ytn ggn tgg gcn lys Gln Tyr Leu Ala Cys Leu Val Leu Ala Met Ala Leu Gly Trp Ala	545	550	1680
ath acn wsn car gay wsn mgn ggn aay aay ath ytn cly gcn ytn gtn Ile Thr Ser Gln Asp Ser Arg Gly Aon Aon Ile Leu His Ala Leu Val	290	295	300	912	aay atg ytn tay tay acn mgn ggn tly car wsn atg ggn atg tay wsn Aon Met Leu Tyr Tyr Thr Arg Gly Phe Gln Ser Met Gly Met Tyr Ser	565	570	1728
acn gtn gcn gar gay tly aar acn car aay gay tly gtn aar mgn atg Thr Val Ala Gln Asp Phe lys Thr Gln Aon Asp Phe Val lys Arg Met	305	310	315	960	gtn atg ath car aar gtn ath ytn cay gay gtn ytn aar tly ytn tly Val Met Ile Gln lys Val Ile Leu His Asp Val Leu lys Leu Phe	580	585	1776
tay gar atg ath ytn ytn mgn wsn ggn aay tgg gar ytn gar acn atg Tyr Asp Met Ile Leu Leu Arg Ser Gly Aon Trp Gln Leu Gln Tyr Met	325	330	335	1008	gtn tay ath ytn tly ytn ytn ggn tly ggn gtn gcn ytn gcn wsn ytn Val Tyr Ile Leu Phe Leu Leu Gly Phe Gly Val Ala Leu Ala Ser Leu	595	600	1824
mgn aay aay gar ggn ytn acn ccn ytn car ytn gcn gcn aar atg ggn Arg Aon Aon Asp Gly Leu Thr Pro Leu Gln Leu Ala Ala lys Met Gly				1056	ath gar aar tgy wsn aar gay aar gar tgy wsn wsn tay ggn wsn Ile Gln lys Cys Ser lys Asp lys lys Asp Cys Ser Ser Tyr Gly Ser			1872

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610

620

615

ttt wsn gay gcn gtn ytn gar ytn ttt aar ytn acn ath ggn ytn ggn
Phe Ser Asp Ala Val Leu Glu Leu Phe Lys Leu Thr Ile Gly Leu Gly
625 630 635

1920

atc acc ccc aca aag aag agt gca ctc ttc ctg gag ata gaa ggg
Ile Thr Pro Thr Lys Lys Ser Ala His Phe Phe Leu Glu Ile Glu Gly
35 40 45

203

gay ytn aay

ath car car aay wsn acn tay acn ath ytn ttt ytn ttt
Asp Leu Aen Ile Thr Tyr Val Ile Leu Thr Phe Val Leu Phe
645 650 655

1968

ttt gaa ccc aac ccc aca gtt gcc aag acc tct cct ctg ttc ttc
Phe Glu Pro Aen Pro Thr Val Ala Lys Thr Ser Pro Pro Val Phe Ser
50 55 60 65

251

ytn ytn ath

acn ttt ytn acn ttt gtn ytn ytn ytn aay atg
Leu Leu Ile Thr Tyr Val Ile Leu Thr Phe Val Leu Leu Aen Met
660 665 670

2016

aag ccc atg gat tcc aac atc cgg cag tgc atc tct ggt aac tgt gat
Lys Pro Met Asp Ser Aen Ile Arg Gln Cys Ile Ser Gly Aen Cys Asp
70 75 80

299

ytn ath gcn

ytn atg ggn gar acn gtn gar aay gtn wsn aar gar wsn
Leu Ile Ala Leu Met Gly Glu Thr Val Glu Aen Val Ser Lys Glu Ser
675 680 685

2064

gac atg gac tcc ccc cag tct cct cag gat gat gta aca gag acc cca
Asp Met Asp Ser Pro Gln Ser Pro Gln Asp Val Thr Glu Thr Pro
85 90 95

347

gar mgn ath

tgg mgn ytn car mgn gcn mgn acn ath ytn gar tty gar
Glu Arg Ile Trp Arg Leu Glu Arg Ala Arg Thr Ile Leu Glu Phe Glu
690 695 700

2112

tcc aat ccc aac agc ccc agt gca cag ctg gcc aag gaa gag cag agg
Ser Aen Pro Aen Ser Pro Ser Ala Gln Leu Ala Lys Glu Gln Arg
100 105 110

395

aar atg ytn

ccn gar tgg ytn mgn wsn mgn ttt mgn atg ggn gar ytn
Lys Met Leu Pro Glu Trp Leu Arg Ser Arg Phe Arg Met Gly Glu Leu
705 710 715 720

2160

agg aaa aag agg cgg ctg aag aag cgc atc ttt gca gcc gtg tct gag
Arg Lys Lys Arg Arg Leu Lys Lys Arg Ile Phe Ala Ala Val Ser Glu
115 120 125

443

tgg aar gtn

gcn gay gar gay tty mgn ytn tgy ytn mgn ath aay gar
Cys Lys Val Ala Asp Glu Asp Phe Arg Leu Cys Leu Arg Ile Aen Glu
725 730 735

2208

ggc tgc gtg gag gag ttg gta gag ttg ctg gtg gag ctg cag gag ctt
Gly Cys Val Glu Glu Leu Val Glu Leu Leu Val Glu Leu Gln Glu Leu
130 135 140 145

491

gtn aar tgg

acn gar tgg aar acn cay gtn wsn tty ytn aay gar gay
Val Lys Trp Thr Glu Trp Lys Thr His Val Ser Phe Leu Aen Glu Asp
740 745 750

2256

tgc agg cgg cgc cat gat gag gat gtg cct gac ttc ctc atg cac aag
Cys Arg Arg Arg His Asp Glu Asp Val Pro Asp Phe Leu Met His Lys
150 155 160

539

ccn ggn ccn

ath mgn mgn acn gcn gay ytn aay aar ath car gay wsn
Pro Gly Pro Ile Arg Arg Thr Ala Asp Leu Aen Lys Ile Gln Asp Ser
755 760 765

2304

ctg acg gcc tcc cag acg ggg aag acc tgc ctg atg aag gcc ttg tta
Leu Thr Ala Ser Asp Thr Gly Lys Thr Cys Leu Met Lys Ala Leu Leu
165 170 175

587

wsn mgn wsn

aay wsn aar acn acn ytn tay gcn tty gay gar ytn gay
Ser Arg Ser Aen Ser Lys Thr Thr Leu Tyr Ala Phe Asp Glu Leu Asp
770 775 780

2352

aac atc aac ccc aac acc aag gag ata gtg cgg atc ctg ctt gcc ttt
Aen Ile Aen Pro Aen Thr Lys Glu Ile Val Arg Ile Leu Leu Ala Phe
180 185 190

635

gar tty ccn

gar acn wsn gtn
Glu Phe Pro Glu Thr Ser Val
785 790

2373

gct gaa gag aac gac atc ctg ggc agg ttc atc aac gcc gag tac aca
Ala Glu Glu Aen Asp Ile Leu Gly Arg Phe Ile Aen Ala Glu Tyr Thr
195 200 205

683

<210> 4

<211> 2432
<212> DNA
<213> Human

731

<220>

<221> CDS
<222> (57)... (2432)

779

<400> 4

gacatcggt gatctcagg caagggttgc cagcaccacc cagaactcca ccagcc atg
Met
1

59

aaa gcc cac

ccc aag gag atg gtg cct ctc atg ggc aag aga gtt get
Lys Ala His Pro Lys Glu Met Val Pro Leu Met Gly Lys Arg Val Ala
5 10 15

107

gcc ccc agt

ggg aac cct gcc gtc ctg cca gag aag agg ccg gcg gag
Ala Pro Ser Gly Aen Pro Ala Val Leu Pro Glu Lys Arg Pro Ala Glu
20 25 30

155

acc tgg cgg

gac tca cga ggc aac aac atc ctt csc gcc ctg gtg acc
Thr Ser Arg Asp Ser Arg Gly Aen Aen Ile Leu His Ala Leu Val Thr
290 300 305

971

gfc ggc gag gac ttc aag acg cag aat gac ttt gtc aag cgc atg tac Val Ala Glu Asp Phe Lys Thr Gln Asn Asp Phe Val Lys Arg Met Tyr 310 315 320	1019
gac atg atc cta ctg cgg agt ggc aac tgg gag ctg gag aac act cgc Asp Met Ile Leu Leu Arg Ser Gly Asn Trp Glu Leu Glu Thr Thr Arg 325 330 335	1067
aac aac gat ggc ctg acg cgg ctg cag ctg ggc ggc aag atg ggc aag Asn Asn Asp Gly Leu Thr Pro Leu Gln Leu Ala Ala Lys Met Gly Lys 340 345 350	1115
gcg gag atc ctg aag tac atc ctg agt cgt gag atc aag gag aag cgg Ala Glu Ile Leu Lys Tyr Ile Leu Ser Arg Glu Ile Lys Glu Lys Arg 355 360 365	1163
ctc cgg agc ctg tcc aag aag ttc acc gac tgg ggc tac gga ccc gtc Leu Arg Ser Leu Ser Arg Lys Phe Thr Asp Trp Ala Tyr Gly Pro Val 370 375 380 385	1211
tca tcc tcc ctg tac gac ctg acc aac gtc gag acc acc acg gac aac Ser Ser Ser Leu Tyr Asp Leu Thr Asn Val Asp Thr Thr Asp Asn 390 395 400	1259
tca gtc ctg gaa atc act gtc tac aac aac aac aac aac aac cgc cat Ser Val Leu Glu Ile Thr Val Tyr Asn Thr Asn Ile Asp Asn Arg His 405 410 415	1307
gag atg ctg acc ctg gag cgg ctg cag acc acg ctg cat atg aag tgg Glu Met Leu Thr Leu Glu Pro Leu His Thr Leu Leu His Met Lys Trp 420 425 430	1355
aag aag ttt gcc aag cac atg ttc ttt ctg tcc ttc tgc ttt tac ttc Lys Lys Phe Ala Lys His Met Phe Phe Leu Ser Phe Cys Phe Tyr Phe 435 440 445	1403
ttc tac aac atc acc ctg acc ctg gtc tgg tac tac cgc ccc cgg gag Phe Tyr Asn Ile Thr Leu Thr Leu Val Ser Tyr Arg Pro Arg Glu 450 455 460 465	1451
gag gag ggc atc cgg cac ccc ctg ggc ctg acg cac aag atg ggg tgg Glu Glu Ala Ile Pro His Pro Leu Ala Leu Thr His Lys Met Gly Trp 470 475 480	1499
ctg cag ctg cta ggg aag atg ttt gtc ctg atc tgg ggc atg tgc atc Leu Gln Leu Leu Gly Arg Met Phe Val Leu Ile Trp Ala Met Cys Ile 485 490 495	1547
tct gtc aaa gag ggc atc gcc atc ttc ctg ctg aga ccc tgg gat ctg Ser Val Lys Glu Gly Ile Ala Ile Phe Leu Leu Arg Pro Ser Asp Leu 500 505 510	1595
cag tcc atc ctg tgg gat gcc tgg ttc cac ttt gtc ttt ttt atc caa Gln Ser Ile Leu Ser Asp Ala Trp Phe His Phe Val Phe Ile Gln 515 520 525	1643
gct gtc ctg gtc ata ctg tct gtc ttc ctg tac tgg ttt gcc tac aaa Ala Val Leu Val Ile Leu Ser Val Phe Leu Tyr Phe Ala Tyr Lys 530 535 540 545	1691
gag tac ctg gcc tgg ctg gtc ggc atg ggc ctg ggc tgg ggc aac Glu Tyr Leu Ala Cys Leu Val Leu Ala Met Ala Leu Gly Trp Ala Asn 550 555 560	1739
atg ctg tac tac acg cgg ggc ttc cag tcc atg ggc atg tac agc gtc Met Leu Tyr Tyr Thr Arg Gly Phe Gln Ser Met Gly Met Tyr Ser Val 565 570 575	1787

atg atc cag aag gtc att tgg cat gat gtc gtc atg aag ttc tgg ttt gta Met Ile Gln Lys Val Ile Leu His Asp Val Leu Lys Phe Leu Phe Val 580 585 590	1835
tat atc gtc ttt tgg ctt gga ttt gga gta gcc tgg ggc tgg ctg atc Tyr Ile Val Phe Leu Leu Glu Phe Gly Val Ala Leu Ala Ser Leu Ile 595 600 605	1883
gag aag tct ccc aaa gac aac aag gac tgc agc tcc tac ggc agc ttc Glu Lys Cys Pro Lys Asp Asn Lys Asp Cys Ser Ser Tyr Gly Ser Phe 610 615 620 625	1931
aac gac gca gtc ctg gaa ctg ttc aag ctg acc ata ggc ctg ggc gac Ser Asp Ala Val Leu Glu Leu Phe Lys Leu Thr Ile Gly Leu Gly Asp 630 635 640	1979
ctg aac atc cag cag aac tcc aag tat ccc att ctg ttt ctg ttc ctg Leu Asn Ile Gln Gln Asn Ser Lys Tyr Pro Ile Leu Phe Leu Phe Leu 645 650 655	2027
ctc atc acc tat gtc atc ctg acc ttt gtc ctg ctg ctc aac atg ctg Leu Ile Thr Tyr Val Ile Leu Thr Phe Val Leu Leu Leu Asn Met Leu 660 665 670	2075
att gct ctg atg ggc gag act gtc gag aac gtc tcc aag gag agc gaa Ile Ala Leu Met Gly Glu Thr Val Glu Asn Val Ser Lys Glu Ser Glu 675 680 685	2123
cgc atc tgg cgc ctg cag aga gcc agc acc atc tgg gag ttt gag aaa Arg Ile Trp Arg Leu Gln Arg Ala Arg Thr Ile Leu Glu Phe Glu Lys 690 695 700 705	2171
atg tta cca gaa tgg ctg agc agc aga ttc cgg atg gga gag ctg tgc Met Leu Pro Glu Trp Leu Arg Ser Arg Phe Arg Met Gly Glu Cys 710 715 720	2219
aaa ctg gcc gag gat gat ttc cga ctg tgt ctg cgg atc aat gag gtc Lys Val Ala Glu Asp Asp Phe Arg Lys Leu Arg Ile Asn Glu Val 725 730 735	2267
aag tgg act gaa tgg aag agc cac gtc tcc ttc ctt aac gaa gac cgg Lys Trp Thr Glu Trp Lys Thr His Val Ser Phe Leu Asn Glu Asp Pro 740 745 750	2315
ggg cct gta aga cga aca gca gat ttc aac aaa atc caa gat tct tcc Gly Pro Val Arg Arg Thr Ala Asp Phe Asn Lys Ile Gln Asp Ser Ser 755 760 765	2363
agg aac aac agc aga aac acc act ctg aat gca ttt gaa gaa gtc ggg gaa Arg Asn Asn Ser Lys Thr Thr Leu Asn Ala Phe Glu Glu Val Glu 770 775 780 785	2411
ttc cgg gaa acc tgg gtc tag Phe Pro Glu Thr Ser Val * 790	2432
<210> 5	
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<212> PRT	
<213> Human	
<400> 5	
Met Lys Ala His Pro Lys Glu Met Val Pro Leu Leu Met Gly Lys Arg Val	
1 5 10 15	
Ala Ala Pro Ser Gly Asn Pro Ala Val Leu Pro Glu Lys Arg Pro Ala	
20 25 30	

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Glu Ile Thr Pro Thr Lys Lys Ser Ala His Phe Phe Leu Glu Ile Glu
 35 40 45
 Gly Phe Glu Pro Asn Pro Thr Val Ala Lys Thr Ser Pro Pro Val Phe
 50 55 60
 Ser Lys Pro Met Asp Ser Asn Ile Arg Gln Cys Ile Ser Gly Asn Cys
 65 70 75
 Asp Asp Met Asp Ser Pro Gln Ser Pro Gln Asp Asp Val Thr Glu Thr
 85 90 95
 Pro Ser Asn Pro Asn Ser Pro Ser Ala Gln Leu Ala Lys Glu Glu Gln
 100 105 110
 Arg Arg Lys Lys Arg Arg Leu Lys Lys Arg Ile Phe Ala Ala Val Ser
 115 120 125
 Glu Gly Cys Val Glu Glu Leu Val Glu Leu Val Glu Leu Gln Glu
 130 135 140
 Leu Cys Arg Arg Arg His Asp Glu Asp Val Pro Asp Phe Leu Met His
 145 150 155
 Lys Leu Thr Ala Ser Asp Thr Gly Lys Thr Cys Leu Met Lys Ala Leu
 165 170 175
 Leu Asn Ile Asn Pro Asn Thr Lys Glu Ile Val Arg Ile Leu Leu Ala
 180 185 190
 Phe Ala Glu Glu Asn Asp Ile Leu Gly Arg Phe Ile Asn Ala Glu Tyr
 195 200 205
 Thr Glu Glu Ala Tyr Glu Gly Gln Thr Ala Leu Asn Ile Ala Ile Glu
 210 215 220
 Arg Arg Gln Gly Asp Ile Ala Ala Leu Leu Ile Ala Ala Gly Ala Asp
 225 230 235
 Val Asn Ala His Ala Lys Gly Ala Phe Phe Asn Pro Lys Tyr Gln His
 245 250 255
 Glu Gly Phe Tyr Phe Gly Glu Thr Pro Leu Ala Leu Ala Cys Thr
 260 265 270
 Asn Gln Pro Glu Ile Val Gln Leu Leu Met Glu His Glu Gln Thr Asp
 275 280 285
 Ile Thr Ser Arg Asp Ser Arg Gly Asn Asn Ile Leu His Ala Leu Val
 290 295 300
 Thr Val Ala Glu Asp Phe Lys Thr Gln Asn Asp Phe Val Lys Arg Met
 305 310 315
 Tyr Asp Met Ile Leu Leu Arg Ser Gly Asn Trp Glu Leu Thr Thr
 325 330 335
 Arg Asn Asn Asp Gly Leu Thr Pro Leu Gln Leu Ala Ala Lys Met Gly
 340 345 350
 Lys Ala Glu Ile Leu Lys Tyr Ile Leu Ser Arg Glu Ile Lys Glu Lys
 355 360 365
 Arg Leu Arg Ser Leu Ser Arg Lys Phe Thr Asp Trp Ala Tyr Gly Pro
 370 375 380
 Val Ser Ser Ser Leu Tyr Asp Leu Thr Asn Val Asp Thr Thr Asp
 385 390 395
 Asn Ser Val Leu Glu Ile Thr Val Tyr Asn Thr Asn Ile Asp Asn Arg
 405 410 415
 His Glu Met Leu Thr Leu Glu Pro Leu His Thr Leu Leu His Met Lys
 420 425 430
 Trp Lys Lys Phe Ala Lys His Met Phe Phe Leu Ser Phe Cys Phe Tyr
 435 440 445
 Phe Phe Tyr Asn Ile Thr Leu Thr Leu Val Ser Tyr Tyr Arg Pro Arg
 450 455 460
 Glu Glu Glu Ala Ile Pro His Pro Leu Ala Leu Thr His Lys Met Gly
 465 470 475
 Trp Leu Gln Leu Leu Gly Arg Met Phe Val Leu Ile Trp Ala Met Cys
 485 490 495
 Ile Ser Val Lys Glu Gly Ile Ala Ile Phe Leu Leu Arg Pro Ser Asp
 500 505 510
 Leu Gln Ser Ile Leu Ser Asp Ala Trp Phe His Phe Val Phe Ile
 515 520 525
 Gln Ala Val Leu Val Ile Leu Ser Val Phe Leu Tyr Leu Phe Ala Tyr
 530 535 540
 Lys Glu Tyr Leu Ala Cys Leu Val Leu Ala Met Ala Leu Gly Trp Ala
 545 550 555
 Asn Met Leu Tyr Tyr Arg Gly Phe Gln Ser Met Gly Met Tyr Ser
 565 570 575

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Val Met Ile Gln Lys Val Ile Leu His Asp Val Leu Lys Phe Leu Phe
 580 585 590
 Val Tyr Ile Val Phe Leu Leu Gly Phe Gly Val Ala Leu Ala Ser Leu
 595 600 605
 Ile Glu Lys Cys Pro Lys Asp Asn Lys Asp Cys Ser Ser Tyr Gly Ser
 610 615 620
 Phe Ser Asp Ala Val Leu Glu Leu Phe Lys Leu Thr Ile Gly Leu Gly
 625 630 635
 Asp Leu Asn Ile Gln Gln Asn Ser Lys Tyr Pro Ile Leu Phe Leu Phe
 640 645 650
 Leu Leu Ile Thr Tyr Val Ile Leu Thr Phe Val Leu Leu Asn Met
 655 660 665
 Leu Ile Ala Leu Met Gly Glu Thr Val Glu Asn Val Ser Lys Glu Ser
 670 675 680
 Glu Arg Ile Trp Arg Leu Gln Arg Ala Arg Thr Ile Leu Glu Phe Glu
 685 690 695
 Lys Met Leu Pro Glu Trp Leu Arg Ser Arg Phe Arg Met Gly Glu Leu
 700 705 710
 Cys Lys Val Ala Glu Asp Asp Phe Arg Leu Cys Leu Arg Ile Asn Glu
 715 720 725
 Val Lys Trp Thr Glu Trp Lys Thr His Val Ser Phe Leu Asn Glu Asp
 730 735 740
 Pro Gly Pro Val Arg Arg Thr Ala Asp Phe Asn Lys Ile Gln Asp Ser
 745 750 755
 Ser Arg Asn Asn Ser Lys Thr Thr Leu Asn Ala Phe Glu Glu Val Glu
 760 765 770
 Glu Phe Pro Glu Thr Ser Val
 775 780 785

<210> 6
 <211> 2373
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (1)... (2373)
 <223> Generic sequence that encompasses all nucleotide
 sequences that encode human TRPV3 having an amino
 acid sequence as shown in SEQ ID NO:5
 n = T or C if after AG

<221> misc feature
 <222> 60,120,180,195,210,231,255,264,294,306,312,384,495,873,882,
 984,1086,1116,1122,1158,1161,1164,1206,1332,1377,1494,1533,1545,1554,1608,
 1713,1728,1821,1860,1863,1872,1878,1944,2055,
 2064,2139,2241,2304,2307,2319,2370
 <223> n = A,T,C or G if after TC;
 n = T or C if after AG

<221> misc feature
 <222> 45,90,219,339,342,351,354,366,441,444,447,564,606,675,678,
 876,885,957,981,1011,1089,1107,1113,1125,1248,1386,1392,
 1461,1527,1701,2070,2079,2088,2136,2142,2148,2187,2199,2271,2274,
 2310
 <223> n = A,T,C or G if after CG;
 n = A or G if after AG

<221> misc feature
 <222> all "n" not specified above
 <223> n = A,T,C or G

<400> 6
 atg aar gcn cay ccn aar gar atg gtn ccn ytn atg ggn aar mgn gtn
 Met Lys Ala His Pro Lys Glu Met Val Pro Leu Met Gly Lys Arg Val
 1 5 10 15 48

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gcn gcn ccn wen ggn aay ccn gcn gtn ytn ccn gar aar mgn ccn gcn
 Ala Ala Pro Ser Gly Aen Pro Ala Val Leu Pro Gln Lys Arg Pro Ala
 20 25 30

gar aeh acn ccn acn aar aar wen gcn cay tcy ytn gar aeh gar
 Gln Ile Thr Pro Thr Lys Lys Ser Ala His Phe Phe Gln Ile Gln
 35 40 45

ggn tly gar ccn aay ccn acn gtn gcn aar acn wen ccn ccn gtn tly
 Gly Phe Gln Pro Aen Pro Thr Val Ala Lys Thr Ser Pro Pro Val Phe
 50 55 60

wen aar ccn atg gay wen aay aeh mgn car tcy ath wen ggn aay tcy
 Ser Lys Pro Met Asp Ser Aen Ile Arg Gln Cys Ile Ser Gly Aen Cys
 65 70 75 80

gay gar atg gay wen ccn car wen ccn car gay gar gtn acn gar acn
 Arg Asp Met Asp Ser Pro Gln Ser Pro Gln Asp Asp Val Thr Gln Thr
 85 90 95

ccn wen aay ccn aay wen ccn wen gcn car ytn gcn aar gar gar car
 Pro Ser Aen Pro Aen Ser Pro Ser Ala Gln Leu Ala Lys Gln Gln
 100 105 110

mgn mgn aar aar mgn ytn aar aar mgn ath tcy gcn gcn gtn wen
 Arg Arg Lys Lys Arg Arg Leu Lys Lys Arg Ile Phe Ala Ala Val Ser
 115 120 125

gar ggn tcy gtn gar gar ytn gtn gar ytn ytn gtn gar ytn car gar
 Gln Gly Cys Val Gln Gln Leu Val Gln Leu Leu Val Gln Gln Gln
 130 135 140

ytn tcy mgn mgn cay gay gar gar gtn ccn gay tcy ytn atg cay
 Leu Cys Arg Arg Arg His Asp Gln Asp Val Pro Asp Phe Leu Met His
 145 150 155 160

aar ytn acn gcn wen gay acn ggn aar acn tcy ytn atg aar gcn ytn
 Lys Leu Thr Ala Ser Asp Thr Gly Lys Thr Cys Leu Met Lys Ala Leu
 165 170 175

ytn aay ath aay ccn aay acn aar gar aeh gtn mgn ath ytn ytn gcn
 Leu Aen Ile Aen Pro Aen Thr Lys Gln Ile Val Arg Ile Leu Leu Ala
 180 185 190

tly gcn gar gar aay gay ath ytn ggn mgn tcy ath aay gcn gar tay
 Phe Ala Gln Gln Aen Asp Ile Leu Gly Arg Phe Ile Aen Ala Gln Tyr
 195 200 205

acn gar gar gcn tay gar ggn car acn gcn ytn aay ath gcn ath gar
 Thr Gln Gln Ala Tyr Gln Gly Gln Thr Ala Leu Aen Ile Ala Ile Gln
 210 215 220

mgn mgn car ggn gay ath gcn gcn ytn ytn ath gcn gcn ggn gcn gay
 Arg Arg Gln Gly Asp Ile Ala Ala Leu Leu Ile Ala Ala Gly Ala Asp
 225 230 235 240

gtn aay gcn cay gcn aar ggn gcn tcy tcy aay ccn aar tay gln his
 Val Aen Ala His Ala Lys Gly Ala Phe Aen Pro Lys Tyr Gln His
 245 250 255

gar ggn tcy tcy tcy ggn gar acn ccn ytn gcn ytn gcn ggn tcy acn
 Gln Gly Phe Tyr Phe Gly Gln Thr Pro Leu Ala Leu Ala Ala Cys Thr
 260 265 270

aay car ccn gar ath gtn car ytn ytn atg gar cay gar acn gay
 Aen Gln Pro Gln Ile Val Gln Leu Leu Met Gln His Gln Thr Asp
 275 280 285

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 Thr Val Ala Gln Asp Phe Lys Thr Gln Aen Asp Phe Val Lys Arg Met
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tay gar atg ath ytn ytn mgn wen ggn aay tgg gar ytn gar acn acn
 Tyr Asp Met Ile Leu Leu Arg Ser Gly Aen Trp Gln Leu Gln Thr Thr
 325 330 335

mgn aay aay gay ggn ytn acn ccn ytn car ytn gcn gcn aar atg ggn
 Arg Aen Aen Asp Gly Leu Thr Pro Leu Gln Leu Ala Ala Lys Met Gly
 340 345 350

aar gcn gar ath ytn aar tay ath ytn wen mgn gar ath aar gar aar
 Lys Ala Ile Ile Leu Lys Tyr Ile Leu Ser Arg Gln Ile Lys Gln Lys
 355 360 365

mgn ytn mgn wen ytn wen mgn aar tcy acn gay tgg gcn tay ggn ccn
 Arg Lys Arg Ser Leu Ser Arg Lys Phe Thr Asp Trp Ala Tyr Gly Pro
 370 375 380

gtn wen wen wen ytn tay gay ytn acn aay gtn gay acn acn gay
 Val Ser Ser Ser Leu Tyr Asp Leu Thr Aen Val Asp Thr Thr Thr Asp
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aay wen gtn gar acn acn gtn tay aay acn aay ath gay aay mgn
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 405 410 415

cay gar atg ytn acn ytn gar ccn ytn cay acn ytn ytn cay atg aar
 His Gln Met Leu Thr Leu Gln Pro Leu His Thr Leu Leu His Met Lys
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Val Met Ile Gln Lys Val Ile Leu His Asp Val Leu Lys Phe Leu Phe
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690 695 700

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Cys Lys Val Ala Glu Asp Asp Phe Arg Leu Cys Leu Arg Ile Asn Glu
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740 745 750

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aaa gcc atc aac acc tct gtc aaa agc aag atc cct tgt gtc gtc gtc lys ala ile aen thr ser val lys ser lys ile pro cys val val val 315	320	325	ggc tgc act ctg gca gcc ttg ggg gcc agc aag ctg ctc aag acc ctg gly cys thr leu ala ala leu gly ala ser lys leu leu lys thr leu 590	595	600
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tat gcg ctg tac aaa gcc ttc agc act aat gag caa gac aag gac aac tyr ala leu tyr lys ala phe ser thr aen glu asp lys asp aen 410	415	420	tgg tat gga gag att tcc cga gac acg aag aac tgg aag att atc ctg trp tyr gly glu ile ser arg asp thr lys aen trp lys ile ile leu 685	690	695
tgg aac gga cag ctg aag ctt ctg ctg gag tgg aac cag ttg gac ctt trp aen gly gln leu lys leu leu leu glu trp aen gln leu 430	435	440	tgt cta ttc att atc ccc tta gtc ggc tgt ggc ctg gta tca ttt aag cys leu phe ile ile pro leu val gly cys gly leu val ser phe arg 700	705	710
ggc agt gat gag atc ttc acc aat gac cgc cgc tgg gag tct gcc gac ala ser asp glu ile phe thr aen asp arg arg trp glu ser ala asp 445	450	455	aag aaa ccc att gac aag cac aag aag ctg ctg tgg tac tat gtc gcc lys lys pro ile asp lys his lys lys leu leu trp tyr val ala 715	720	725
ctt cag gag gtc atg ttc acg gct ctg ata aag gac aga ccc aag ttt leu gln glu val met phe thr ala leu ile lys asp arg pro lys phe 460	465	470	ttc ttc aag tgg ccc ttc gtc gtc ttc tcc tgg aac gtc gtc ttc tac phe phe thr ser pro phe val val phe ser trp aen val val phe 730	735	740
ggc cgc ccc ttt ctg gag aat ggc ctg aat ctg cag aag ttt ctg acc val arg leu phe leu glu aen gly leu aen leu lys phe leu thr 475	480	485	acc gcc ttc ctg ctg ctg ttt gcc tat gtc ctg ctg atg gac ttc cac ile ala phe leu leu leu phe ala tyr val leu leu met asp phe his 750	755	760
aat gaa gtc ctc aca gag ctg ttc tcc acc cac ttc agc acc cta gtc aen glu val leu thr glu leu phe ser thr his phe ser thr leu val 1962			tca gtc cca cac acc ccc gag ctg atc ctg tcc gcc ctg gtc ttc gtc ser val pro his thr pro glu leu ile leu tyr ala leu val phe val 2778		

21/75

22/75

765

775

1035

1040

1045

ctc ttc tgt gat gaa gtg agg cag tgg tac atg aac gga gtg aat tat 2826
Leu Phe Cys Asp Glu Val Arg Gln Trp Tyr Met Asn Gly Val Asn Tyr
780 790

ttc acc gac cta tgg aac gtt atg gac acc ctg gga ctc ttc tac ttc 2874
Phe Thr Asp Leu trp Asn Val Met Asp Thr Leu Gly Leu Phe Tyr Phe
795 800 805

ata ggc ggt att gta ttc cgg ctc cac tct aat aaa agc tgc ttg 2922
ile Ala Gly ile Val Phe Arg Leu His Ser Ser Asn Lys Ser Ser Leu
810 815 820 825

tac tct ggg cgc gtc atc ttc tgt ctg gat tac att ata ttc acg cta 2970
Tyr Ser Gly Arg Val ile Phe Cys Leu Asp Tyr ile ile Phe Thr Leu
830 835 840

agg ctc atc cac att ttc acc gtc agc agg aac ttg gga ccc aag att 3018
Arg Leu ile His ile Phe Thr Val Ser Arg Asn Leu Gly Pro Lys ile
845 850 855

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ile Met Leu Gln Arg Met Leu ile Asp Val Phe Phe Leu Phe Leu
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Phe Ala Val Trp Met Val Ala Phe Gly Val Ala Arg Gln Gly ile Leu
875 880 885

agg naa aat gaa cag cgc tgg aga ttc ctc cgc tct gtc atc tat 3162
Arg Gln Asn Glu Gln Arg Trp Arg Trp ile Phe Arg Ser Val ile Tyr
890 895 900 905

gag ccc tac ctg gcc atg ttt ggc cag gtt ccc agt gac gtg gat agt 3210
Glu Pro Tyr Leu Ala Met Phe Gly Gln Val Pro Ser Asp Val Asp Ser
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acc aca tat gac ttc tcc cac tgt acc ttc tgc gga aat gag tcc aag 3258
Thr Thr Tyr Asp Phe Ser His Cys Thr Phe Ser Gly Asn Glu Ser Lys
925 930 935

cca ctg tgt gtc gag ctg gat gag cac aac ctg ccc cgc ttc cct gag 3306
Pro Leu Cys Val Glu Leu Asp Glu His Asn Leu Pro Arg Phe Pro Glu
940 945 950

tgg atc acc att ccg ctg gtc tgc atc tac atg ctc tcc acc aat atc 3354
Trp ile Thr ile Pro Leu Val Cys ile Tyr Met Leu Ser Thr Asn ile
955 960 965

ctt ctg gtc aac ctc ctg gtc gcc atg ttt ggc tac acg gta ggc att 3402
Leu Leu Val Asn Leu Leu Val Ala Met Phe Gly Tyr Thr Val Gly ile
970 975 980 985

gta cag gag aac aac gac cag gtc tgg aaa ttc cag cgg tac ttc ctg 3450
Val Gln Glu Asn Asn Asp Gln Val Trp Lys Phe Gln Arg Tyr Phe Leu
990 995 1000

gtg cag gag tac tgc aac cgc cta aac atc ccc ttc ccc ttc gtt gtc 3498
Val Gln Glu Tyr Cys Asn Arg Leu Asn ile Pro Phe Pro Phe Val Val
1005 1010 1015

ttc gct tat ttc tac atg gtc agc aag tgc ttc aac tgc tgc tgt 3546
Phe Ala Tyr Phe Tyr Met Val Val Lys Lys Cys Phe Lys Cys Cys
1020 1025 1030

aaa gag aag aat atg gag tct aat gcc tgc tgt ttc aga aat gag gac 3594
Lys Glu Lys Asn Met Glu Ser Asn Ala Cys Phe Arg Asn Glu Asp
1035

aat gag act ttg gcg tgg gag ggt gtc atg aag gag aat tac ctt gtc 3642
Asn Glu Thr Leu Ala Trp Glu Gly Val Met Lys Glu Asn Tyr Leu Val
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Lys ile Asn Thr Lys Ala Asn Asp Asn Ser Glu Glu Met Arg His Arg
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Phe Arg Gln Leu Asp Ser Lys Leu Asn Asp Leu Lys Ser Leu Leu Lys
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gag att gct aat aac atc aag taa ggc tgg cgc gat gct tgg gggg aga aac aaaa 3792
Glu ile Ala Asn Asn ile Lys *
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35 40 45
Phe Lys Lys Arg Glu Cys Val Phe Phe Thr Arg Asp Ser Lys Ala Met
50 55 60
Glu Asn ile Cys Lys Cys Gly Tyr Ala Gln Ser Gln His ile Glu Gly
65 70 75 80
Thr Gln ile Asn Gln Asn Glu Lys Trp Asn Tyr Lys His Thr Lys
85 90 95
Glu Phe Pro Thr Asp Ala phe Gly Asp ile Gln Phe Glu Thr Leu Gly
100 105 110
Lys Lys Gly Lys Tyr Leu Arg Leu Ser Cys Asp Thr Asp Ser Glu Thr
115 120 125
Leu Tyr Glu Leu Leu Thr Gln His Trp His Leu Lys Thr Pro Asn Leu
130 135 140
Val ile Ser Val Thr Gly Gly Ala Lys Asn Phe Ala Leu Lys Pro Arg
145 150 155 160
Met Arg Lys ile Phe Ser Arg Leu ile Tyr ile Ala Gln Ser Lys Gly
165 170 175
Ala Trp ile Leu Thr Gly Gly Thr His Tyr Gly Leu Met Lys Tyr ile
180 185 190
Gly Glu Val Val Arg Asp Asn Thr ile Ser Arg Asn Ser Glu Glu Asn
195 200 205
ile Val Ala ile Gly ile Ala Ala Trp Gly Met Val Ser Asn Arg Asp
210 215 220
Thr Leu ile Arg Ser Cys Asp Asp Glu Gly His Phe Ser Ala Gln Tyr
225 230 235
ile Met Asp Asp Phe Thr Arg Asp Pro Leu Tyr ile Leu Asp Asn Asn
240 245 250 255
His Thr His Leu Leu Leu Val Asp Asn Gly Cys His Gly His Pro Thr
260 265 270
Val Glu Ala Lys Leu Arg Asn Gln Leu Glu Lys Tyr ile Ser Glu Arg
275 280 285
Thr Ser Gln Asp Ser Asn Tyr Gly Gly Lys ile Pro ile Val Cys Phe

290 Ala Gln Gly Gly Arg Gln Thr Leu Lys Ala Ile Asn Thr Ser Val
 305 310 315 320
 Lys Ser Lys Ile Pro Cys Val Val Val Gln Gly Ser Gly Gln Ile Ala
 325 330 335
 Asp Val Ile Ala Ser Leu Val Gln Val Asp Val Leu Thr Ser Ser
 340 345 350
 Met Val Lys Gln Lys Leu Val Arg Phe Leu Pro Arg Thr Val Ser Arg
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 Leu Pro Gln Gln Gln Ile Ile Ser Trp Ile Lys Trp Leu Lys Gln Ile
 370 375 380
 Leu Gln Ser Ser His Leu Leu Thr Val Ile Lys Met Gln Ala Gly
 385 390 395 400
 Asp Gln Ile Val Ser Asn Ala Ile Ser Tyr Ala Leu Tyr Lys Ala Phe
 405 410 415
 Ser Thr Asn Gln Asp Lys Asp Asn Trp Asn Gly Gln Leu Lys Leu
 420 425 430
 Leu Leu Gln Trp Asn Gln Leu Asp Leu Ala Ser Asp Ile Phe Thr
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 Asn Asp Arg Arg Trp Gln Ser Ala Asp Leu Gln Gln Val Met Phe Thr
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 Ala Leu Ile Lys Asp Arg Pro Lys Phe Val Arg Leu Phe Leu Gln Asn
 465 470 475 480
 Gly Leu Asn Leu Gln Lys Phe Leu Thr Asn Gln Val Leu Thr Gln Leu
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 Phe Ser Thr His Phe Ser Thr Leu Val Tyr Arg Asn Leu Gln Ile Ala
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 Lys Asn Ser Tyr Asn Asp Ala Leu Leu Thr Phe Val Trp Lys Leu Val
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 Ala Asn Phe Arg Arg Ser Phe Trp Lys Gln Asp Arg Ser Arg Gln
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 Leu Gln Ala Leu Phe Ile Trp Ala Ile Leu Gln Asn Lys Lys Gln Leu
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 Ser Lys Val Ile Trp Gln Gln Thr Lys Gly Cys Thr Leu Ala Ala Leu
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 Gly Ala Ser Lys Leu Leu Lys Thr Leu Ala Lys Val Lys Asn Asp Ile
 595 600 605
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 610 615 620
 Ala Val Gln Leu Phe Thr Gln Cys Tyr Ser Asn Asp Gln Asp Leu Ala
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 Gln Gln Leu Leu Val Tyr Ser Cys Gln Ala Trp Gly Gly Ser Asn Cys
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 Leu Gln Leu Ala Val Gln Ala Thr Asn Gln His Phe Ile Ala Gln Pro
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 Asp Thr Lys Asn Trp Lys Ile Ile Leu Cys Leu Phe Ile Ile Pro Leu
 690 695 700
 Val Gly Cys Gly Leu Val Ser Phe Arg Lys Pro Ile Asp Lys His
 705 710 715 720
 Lys Lys Leu Leu Trp Tyr Tyr Val Ala Phe Phe Thr Ser Pro Phe Val
 725 730 735
 Val Phe Ser Trp Asn Val Val Phe Tyr Ile Ala Phe Leu Leu Phe
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 Leu Ile Leu Tyr Ala Leu Val Phe Val Leu Phe Cys Asp Gln Val Arg
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 Gln Trp Tyr Met Asn Gly Val Asn Tyr Phe Thr Asp Leu Trp Asn Val
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 Met Asp Thr Leu Gly Leu Phe Tyr Phe Ile Ala Gly Ile Val Phe Arg
 805 810 815
 Leu His Ser Ser Asn Lys Ser Ser Leu Tyr Ser Gly Arg Val Ile Phe
 820 825 830
 Cys Leu Asp Tyr Ile Ile Phe Thr Leu Arg Leu Ile His Ile Phe Thr

835 Val Ser Arg Asn Leu Gly Pro Lys Ile Ile Met Leu Gln Arg Met Leu
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 855 860 865
 865 Phe Gly Val Ala Ser Gln Gly Ile Leu Arg Gln Asn Gln Arg Trp
 870 875 880
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 890 895 900
 905 Gly Gln Val Pro Ser Asp Val Asp Ser Thr Thr Tyr Asp Phe Ser His
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 925 Cys Thr Phe Ser Gly Asn Gln Ser Lys Pro Leu Cys Val Gln Leu Asp
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 945 Gln His Asn Leu Pro Arg Phe Pro Gln Trp Ile Thr Ile Pro Leu Val
 950 955 960
 965 Cys Ile Tyr Met Leu Ser Thr Asn Ile Leu Leu Val Asn Leu Leu Val
 970 975 980
 985 Ala Met Phe Gly Tyr Thr Val Gly Ile Val Gln Gln Asn Asn Asp Gln
 990 995 1000
 1005 Val Trp Lys Phe Gln Arg Tyr Phe Leu Val Gln Gln Tyr Cys Asn Arg
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<213> Artificial Sequence

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<223> Generic sequence that encompasses all nucleotide sequences that encode mouse TRPM8 having an amino acid sequence as shown in SEQ ID NO:8

<221> CDS

<222> (1)... (3312)

<221> misc feature

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 1215,1227,1251,1329,1365,1494,1506,1545,1602,1623,1626,1733,1785,
 1842,1902,1941,1962,2037,2061,2133,2199,2217,2286,2457,2460,2469,2472,
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 <223> n = A,T,C or G if after TC;
 n = T or C if after AG

<221> misc feature

<222> 21,33,39,42,66,90,156,177,357,480,486,501,591,609,669,684,
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 1586,1599,1620,1629,1674,1872,2064,2139,2352,2448,2487,2526,2553,2586,
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 n = T or C if after AG

<221> misc feature

<222> all "n" not specified above
 <223> n = A,T,C or G

<400> 9 atg wsn ttc gar ggn gcn mgn ytn wsn atg mgn wsn mgn aay ggn 48 Met Ser Phe Glu Gly Ala Arg Leu Ser Met Arg Ser Arg Arg Asn Gly 15 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530 535 540
 gny ytn gar ytn gar ytn cay gar ytn gcn wsn ytn acn acn mgn cay ccn
 App Leu Asp Val Glu Leu His Asp Ala Ser Leu Thr Arg His Pro 560
 545 550 555
 ytn car gcn ytn tly ath tgg gcn ath ytn car bay aar aar gar ytn
 Leu Glu Ala Leu Phe Ile Tyr Ala Ile Leu Glu Asn Lys Lys Glu Leu 575
 565 570
 wsn aar gtn ath tgg gar car acn aar gar tgy acn ytn gcn gcn ytn
 Ser Lys Val Ile Tyr Glu Glu Thr Lys Gly Cys Thr Leu Ala Ala Leu 590
 580 585
 ggn gcn wsn aar ytn ytn aar acn ytn gcn aar gtn aar aay gar ath
 Gly Ala Ser Lys Leu Leu Lys Thr Leu Ala Lys Val Lys Asn Asp Ile 605
 595 600
 aay gcn gcn ggn gar wsn gar gar ytn gcn aay gar tay gar acn mgn
 Asn Ala Ala Gly Glu Ser Glu Glu Leu Ala Asn Tyr Glu Thr Arg 620
 610 615
 gcn gtn gar ytn tly acn gar tgy tay wsn aay gar gar ytn gcn
 Ala Val Glu Leu Phe Thr Glu Cys Tyr Ser Asn Asp Glu Asp Leu Ala 630
 625 635
 gar car ytn ytn gtn tay wsn tgy gar gar tgg ggn ggn wsn aay tgy
 Glu Glu Leu Leu Val Tyr Ser Cys Glu Ala Tyr Gly Gly Ser Asn Cys 650
 645 655
 ytn gar ytn gcn gtn gar gcn acn gar car cay tly ath gcn car ccn
 Leu Glu Leu Ala Val Glu Ala Thr Asp Glu His Phe Ile Ala Glu Pro 670
 660 665
 ggn gtn car aay tly ytn wsn aar car tgg tay ggn gar ath wsn mgn
 Gly Val Glu Asn Phe Leu Ser Lys Glu Tyr Tyr Gly Glu Ile Ser Arg 680
 675 685
 gar acn aar aay tgg aar ath ath ytn tgy ytn tly ath acn ytn
 Asp Thr Lys Asn Tyr Lys Ile Ile Leu Cys Leu Phe Ile Ile Pro Leu 700
 690 695
 gtn ggn tgy ggn ytn gtn wsn tly mgn aar aar ccn ath gar aar cay
 Val Gly Cys Gly Leu Val Ser Phe Arg Lys Lys Pro Ile Asp Lys His 710
 705 715
 aar aar ytn ytn tgg tay tay gcn gcn tly tly acn wsn ccn tly gtn
 Lys Lys Leu Leu Tyr Tyr Tyr Val Ala Phe Phe Thr Ser Pro Phe Val 730
 725 735
 gtn tly wsn tgg aay gtn gtn tly tay ath gcn tly ytn ytn tly
 Val Phe Ser Tyr Asn Val Val Phe Tyr Ile Ala Phe Leu Leu Phe 740
 745 750
 gcn tay gtn ytn ytn atg gar tly cay wsn gtn ccn cay acn ccn gar
 Ala Tyr Val Leu Leu Met Asp Phe His Ser Val Pro His Thr Pro Glu 760
 755 765
 ytn ath ytn tgy gcn ytn gtn tly gtn ytn tly tgy gar gar gtn mgn
 Leu Ile Leu Tyr Ala Leu Val Phe Val Phe Cys Asp Glu Val Arg 780
 770 785
 car tgg tay atg aay ggn gtn aay tay tly acn gar ytn tgg aay gtn
 Glu Thr Tyr Met Asn Gly Val Asn Tyr Phe Thr Asp Leu Thr Asn Val 790
 785 795
 atg gar acn ytn ggn ytn tly tay tly ath gcn ggn ath gtn tly mgn
 Met Asp Thr Leu Gly Leu Phe Tyr Phe Ile Ala Gly Ile Val Phe Arg 800

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530 535 540
 ytn cay wsn wsn aay aar wsn ytn tay wsn ggn mgn gtn ath tly
 Leu His Ser Ser Asn Lys Ser Ser Leu Tyr Ser Gly Arg Val Ile Phe 820
 805 810 815
 tgy ytn gar tay ath ath tly acn ytn mgn ytn ath cay ath tly acn
 Cys Leu Asp Tyr Ile Ile Phe Thr Leu Arg Leu Ile His Ile Phe Thr 840
 835 845
 gtn wsn mgn aay ytn ggn ccn aar ath ath atg ytn car mgn atg ytn
 Val Ser Arg Asn Leu Gly Pro Lys Ile Ile Met Leu Leu Arg Met Leu 850
 855 860
 ath gar gtn tly tly tly ytn tly ytn tly gcn gtn tgg atg gtn gcn
 Ile Asp Val Phe Phe Leu Leu Phe Leu Phe Ala Val Tyr Met Val Ala 870
 865 875
 tly ggn gtn gcn mgn car ggn ath ytn mgn car aay gar car mgn tgg
 Phe Gly Val Ala Arg Glu Gly Ile Leu Arg Glu Asn Glu Glu Arg Tyr 885
 890 895
 mgn tgg ath tly mgn wsn gtn ath tay gar ccn tay ytn gcn atg tly
 Arg Tyr Ile Phe Arg Ser Val Ile Tyr Glu Pro Tyr Leu Ala Met Phe 905
 910 915
 ggn car gtn ccn wsn gar ytn gar wsn acn acn tay gar tly wsn cay
 Gly Glu Val Pro Ser Asp Val Asp Ser Thr Thr Tyr Asp Phe Ser His 920
 915 925
 tgy acn tly wsn ggn aay gar wsn aar ccn ytn tgy gtn gar ytn gar
 Cys Thr Phe Ser Gly Asn Glu Ser Lys Pro Leu Cys Val Glu Leu Asp 930
 935 940
 gar cay aay ytn ccn mgn tly ccn gar tgg ath acn acn ytn gtn
 Glu His Asn Leu Pro Arg Phe Pro Glu Tyr Ile Thr Ile Pro Leu Val 950
 945 955
 tgy ath tay atg ytn wsn acn aay ath ytn ytn gtn aay ytn ytn gtn
 Cys Ile Tyr Met Leu Ser Thr Asn Ile Leu Leu Val Asn Leu Val 965
 970 975
 gcn atg tly ggn tay acn gtn ggn ath gtn car gar aay aay gar car
 Ala Met Phe Gly Tyr Thr Val Gly Ile Val Glu Glu Asn Asp Glu 980
 985 990
 gtn tgg aar tly car mgn tay tly ytn gtn car gar tay tgy aay mgn
 Val Tyr Lys Phe Glu Arg Tyr Phe Leu Val Glu Glu Tyr Cys Asn Arg 995
 1000 1005
 ytn aay ath ccn tly ccn tly gtn gtn tly gcn tay tly atg gtn
 Leu Asn Ile Pro Phe Pro Phe Val Val Phe Ala Tyr Phe Tyr Met Val 1010
 1015 1020
 gtn aar aar tgy tly aar tgy tgy aar gar aar aay atg gar wsn
 Val Lys Lys Cys Phe Lys Cys Cys Lys Lys Asn Met Glu Ser 1030
 1025 1035
 aay gcn tgy tgy tly mgn aay gar gar aay acn ytn gcn tgg gar
 Asn Ala Cys Cys Phe Arg Asn Glu Asp Asn Glu Thr Leu Ala Tyr Glu 1045
 1050 1055
 ggn gtn atg aar gar aay tay ytn gtn aar ath aay acn aar gcn aay
 Gly Val Met Lys Glu Asn Tyr Leu Val Lys Ile Asn Thr Lys Ala Asn 1060
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 gar aay wsn gar gar atg mgn cay mgn tly mgn car ytn gar wsn aar
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PCT/EP02/06520

PCT/EP02/06520

29/75

30/75

1085

1075

1080

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Met Pro Leu Pro His Lys Ser Gly Gin Lys Ser Leu Arg Ser Tyr Phe
1 5 10 15

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Val Phe Ser Ile Gln Val Ser Val Ile Gln Ile Lys Gly Thr Glu Ser

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pro gly phe ala trp trp ala phe ser gly pro leu phe arg phe leu

cct ttc tcc tgg ttg ctg gcc ttg gag ctg acc gtg gtg ctg aca gga 252
 pro phe ser val leu leu ala leu glu leu thr val val leu thr gly 50
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Val Trp Arg Leu Leu Arg Pro Cys Tyr His Cys Val Tyr Cys Gly Pro 80
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Ala Ala Ser Ala His Leu Phe Ile Lys Gln Trp Leu Asp Gly Trp Arg

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Met Gln Val Asp Arg Arg Arg Gly Ala Cys Arg Ser Lys Gly Leu Val

cag gtt gaa ggg gct aca cag gca ggt gag cac ttg ctc agc ctg ggc
Gln Val Glu Gly Ala Thr Gln Ala Gly Glu His Leu Ser Leu Gly

att gtg ggg cat ctc cct gaa gaa atg atg agt gag ctg agc ctg gag 492
ile val gly his leu leu pro glu glu met met ser glu leu ser leu glu

139	140
gat gag cag gag atg aca gct gga ggg gta tgg gga aga ggg ctc tgg	540
acc gln gln gln gln met thr al a gln gln val tgg gln tgg gln	

aca gaa gaa aag atg tcc ttt cgg gca gcc agg ctc agc atg agg aac 588
145 150 155 160

aga agg aat gac act ctg gac agc acc cgg acc ctg tac tcc agc gcg 636

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Ser Arg Ser Thr Asp Leu Ser Tyr Ser Glu Ser Asp Leu Val Asn Phe
195 200 205

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 ile gln ala asn phe lys lys arg glu cys val phe phe ile lys asp 733
 210 215 220

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Ser Lys Ala Thr Glu Asn Val Cys Lys Cys Gly Tyr Ala Gln Ser Gln 785
225 226 227 228 229 230 231 232 233 234

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His Met Glu Gly Thr Gln Ile Asn Gln Ser Glu Lys Trp Asn Tyr Lys

aaa cac acc aag gaa ttt cct acc gac gcc ttt ggg gat att cag ttt
lys his thr lys glu phe pro thr asp ala phe gly asp ile gln phe

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gag aca ctg ggg aag aaa ggg aag tat ata cgt ctg tcc tgc gag acg		924
Glu Thr Ileu Gly ILeu ILeu Gly ILeu Thr Tla Met Leu Ser Cys Met		

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aca ccc aac ctg gtc att tct gtg acc ggg ggc gcc aag aac ttc gcc 1020

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Met Lys Tyr Ile Gly Glu Val Val Arg Asp Asn Thr Ile Ser Arg Ser
355 360 365

Ser Glu Glu Asn Ile Val Ala Ile Gly Ile Ala Trp Gly Met Val
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Ser Asn Arg Asp Thr Leu Ile Arg Asn Cys Asp Ala Glu Gly Tyr Phe
 385 390 395 400

Leu Ala Gln Tyr Leu Met Asp Asp Phe Thr Arg Asp Pro Leu Tyr Ile
405 410 415

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485 490 495
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Gly Gln Ile Ala Asp Val Ile Ala Ser Leu Val Glu Val Asp Ala
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Leu Thr Ser Ser Ala Val Lys 520 Phe Leu Pro Arg
515 525
aag gtc tcc cgg ctc cct gag gag gag act gag agt tgg atc aac tgg
Thr Val Ser Arg Leu Pro Glu Glu Thr Glu Ser Trp Ile Lys Trp
530 535 540
ccc aaa gaa att ctc gaa tgt tct cac tta tta aca gtt att aaa atg
Leu Lys Glu Ile Leu Glu Cys Ser His Leu Thr Val Ile Lys Met
545 550 555 560
gaa gaa gct ggg gat gaa att gtc agc aat ggc tcc tac gct cta
Glu Glu Ala Gly Asp Glu Ile Val Ser Aaa Ala Ile Ser Tyr Ala Leu
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Tyr Lys Ala Phe Ser Thr Ser Glu Gln Aap Lys Aap Aaa Glu
580 585 590
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Gln Leu Lys Leu Leu Glu Trp Aaa Gln Leu Aap Leu Ala Aaa Aap
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Val Met Phe Thr Ala Leu Ile Lys Aap Arg Pro Lys Phe Val Arg Leu
625 630 635 640
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ctc act gaa ctc ttc tcc aac cac ttc agc acg ctt gtc tac cgg aat
Leu Thr Glu Leu Phe Ser Aaa His Phe Ser Thr Leu Val Tyr Arg Aaa
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705 710 715 720
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Thr Arg His Pro Leu Gln Ala Leu Phe Ile Trp Ala Ile Leu Aaa
725 730 735
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2316

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740 745 750
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Leu Ala Ala Leu Gly Ala Ser Lys Leu Leu Lys Thr Leu Ala Lys Val
755 760 765
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Tyr Glu Thr Arg Ala Val Glu Leu Phe Thr Glu Cys Tyr Ser Ser Aap
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Gly Ser Aaa Cys Leu Glu Leu Ala Val Glu Ala Thr Asp Gln His Phe
820 825 830
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Ile Ala Gln Pro Gly Val Val Aaa Phe Leu Ser Lys Trp Tyr Gly
835 840 845
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Glu Ile Ser Arg Aap Thr Lys Aaa Trp Lys Ile Ile Leu Cys Leu Phe
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865 870 875 880
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Val Aap Lys His Lys Lys Leu Leu Trp Tyr Tyr Val Ala Phe Thr
885 890 895
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Ser Pro Phe Val Val Phe Ser Trp Aaa Val Val Phe Tyr Ile Ala Phe
900 905 910
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Leu Leu Leu Phe Ala Tyr Val Leu Leu Met Asp Phe His Ser Val Pro
915 920 925
cac ccc ccc ggg ctc gtc ctc tac tgc ctc gtc ttt gtc ctc ttc tgt
His Pro Pro Glu Leu Val Leu Tyr Ser Leu Val Phe Val Leu Phe Cys
930 935 940
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Aap Glu Val Arg Gln Trp Tyr Val Aaa Glu Val Aaa Tyr Phe Thr Aap
945 950 955 960
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965 970 975
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980 985 990
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Arg Val Ile Phe Cys Leu Aap Tyr Ile Ile Phe Thr Leu Arg Leu Ile
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3132

33/75

34/75

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 Asp Phe Ala His Cys Thr Phe Thr Gly Asn Gln Ser Lys Pro Leu Cys
 1095 1100
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 1125 1130 1135
 aac ctg ctg gtc gcc atg ttt ggc tac acg gtc ggc acc gtc cag gag
 Asn Leu Leu Val Ala Met Phe Gly Tyr Thr Val Gly Thr Val Gln Gln
 1140 1145 1150
 aac aat gac cag gtc tgg aag ttc cag agg tac ttc ctg gtc cag gag
 Asn Asn Asp Gln Val Trp Lys Phe Gln Arg Tyr Phe Leu Val Gln Gln
 1155 1160 1165 1170
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 Tyr Cys Ser Arg Leu Asn Ile Pro Phe Pro Phe Ile Val Phe Ala Tyr
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 ttc tac atg gtc gtc aag aag tgc ttc aag tgt tgc tgc aag gag aaa
 Phe Tyr Met Val Val Lys Lys Cys Phe Lys Cys Cys Lys Gln Lys
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 Asn Met Gln Ser Ser Val Cys Cys Phe Lys Asn Gln Asp Asn Gln Thr
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 Leu Ala Trp Gln Gly Val Met Lys Gln Asn Tyr Leu Val Lys Ile Asn
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 Thr Lys Ala Asn Asp Thr Ser Gln Gln Met Arg His Arg Phe Arg Gln
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 1255 1260
 aat aaa atc aaa taa
 Asn Lys Ile Lys *
 1265

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 Pro Phe Ser Val Leu Leu Ala Leu Gln Leu Thr Val Val Leu Thr Gly
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 Val Trp Arg Leu Leu Arg Pro Cys Tyr His Cys Val Tyr Cys Gly Pro
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 100 105 110
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 115 120 125
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 Lys His Thr Lys Gln Phe Pro Thr Asp Ala Phe Gly Asp Ile Gln Phe
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 305 310 315 320
 Leu Lys Pro Arg Met Arg Lys Ile Phe Ser Arg Leu Ile Tyr Ile Ala
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 Met Lys Tyr Ile Gly Gln Val Val Arg Asp Asn Thr Ile Ser Arg Ser
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 405 410 415
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 Gly His Pro Thr Val Gln Ala Lys Leu Arg Asn Gln Leu Gln Lys Tyr
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 Ile Ser Gln Arg Thr Ile Gln Asp Ser Asn Tyr Gly Gly Lys Ile Pro
 450 455 460
 Ile Val Cys Phe Ala Gln Gly Gly Lys Gln Thr Leu Lys Ala Ile
 465 470 475 480
 Asn Thr Ser Ile Lys Asn Lys Ile Pro Cys Val Val Gln Gly Ser
 485 490 495

Gly Gln Ile Ala Asp Val Ile Ala Ser Leu Val Glu Val Glu Asp Ala
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 Leu Thr Ser Ala Val Lys Glu Leu Val Arg Phe Leu Pro Arg
 515 520 525
 Thr Val Ser Arg Leu Pro Glu Glu Thr Glu Ser Trp Ile Lys Trp
 530 535 540
 Leu Lys Glu Ile Leu Glu Cys Ser His Leu Leu Thr Val Ile Lys Met
 545 550 555
 Glu Glu Ala Gly Asp Glu Ile Val Ser Asn Ala Ile Ser Tyr Ala Leu
 565 570 575
 Tyr Lys Ala Phe Ser Thr Ser Glu Gln Asp Lys Asp Asn Trp Asn Gly
 585 590 595
 Gln Leu Lys Leu Leu Glu Trp Asn Gln Leu Asp Leu Ala Asn Asp
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 Glu Ile Phe Thr Asn Asp Arg Arg Trp Glu Ser Ala Asp Leu Gln Glu
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 Val Met Phe Thr Ala Leu Ile Lys Asp Arg Pro Lys Phe Val Arg Leu
 630 635 640
 Phe Leu Glu Asn Gly Leu Asn Leu Arg Lys Phe Leu Thr His Asp Val
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 660 665 670
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 675 680 685
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 Thr Arg His Pro Leu Gln Ala Leu Phe Ile Trp Ala Ile Leu Gln Asn
 720 725 730
 Lys Lys Glu Leu Ser Lys Val Ile Trp Glu Gln Thr Arg Gly Cys Thr
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 Gly Ser Asn Cys Leu Glu Leu Ala Val Glu Ala Thr Asp Gln His Phe
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 915 920 925
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 596,1845,1848,1902,1917,1947,2013,2068,2091,2100,2112,2121,2166,2247,2364,
 2556,2631,2844,2940,2979,3018,3045,3078,3147,3162,3177,3183,3195,3342,
 3486,3516,3729,3735,3741
 <223> n = A,T,C or G if after CG;
 n = A or G if after AG
 <221> misc.feature
 <222> all "n" not specified above
 <223> n = A,T,C or G

[illegible]

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glu	thr	leu	gñy	lys	lys	gñy	lys	tyr	ile	arg	leu	ser	cys	asp	thr	
		275						280								
gay	gcn	gar	ath	ytñ	tay	gar	ytñ	ytñ	acn	car	cay	tgg	cay	ytñ	aar	912
asp	ala	glu	ile	leu	tyr	glu	leu	leu	thr	gñn	his	trp	his	leu	lys	
		290						295				300				
acn	cnñ	aay	ytñ	gtn	ath	wen	gtn	acn	ggn	ggn	gcn	aar	aay	ttñ	gcn	960
thr	pro	asn	leu	val	ile	ser	val	thr	gñy	glu	ala	lys	asn	pñe	ala	
		305						310				315			320	
ytñ	aar	cnñ	mgn	atg	mgn	aar	ath	ttñ	wen	mgn	ytñ	ath	tay	ath	gcn	1008
leu	lys	pro	arg	met	arg	lys	ile	pñe	ser	arg	leu	ile	tyr	ile	ala	
			325								330				335	
car	wen	aar	ggn	gcn	tgg	ath	ytñ	acn	ggn	ggn	acn	cay	tay	ggn	ytñ	1056
gñn	ser	lys	gñy	ala	trp	ile	leu	thr	gñy	glu	thr	his	tyr	gñy	leu	
			340					345							350	
atg	aar	tay	ath	ggn	gar	gtn	gtn	mgn	gay	aay	acn	ath	wen	mgn	wen	1104
met	lys	tyr	ile	gñy	glu	val	val	arg	asp	asn	thr	ile	ser	arg	ser	
		355						360							365	
wen	gar	gar	aay	ath	gtn	gcn	ath	ggn	ath	gcn	gcn	tgg	ggn	atg	gtn	1152
ser	glu	glu	asn	ile	val	ala	ile	gñy	ile	ala	ala	trp	gñy	met	val	
		370						375							380	
wen	aay	mgn	gay	acn	ytñ	ath	mgn	aay	tgy	gay	gcn	gar	ggn	tay	ttñ	
ser	asn	arg	asp	thr	leu	ile	arg	asn	cys	asp	ala	glu	gñy	tyr	pñe	
		385						390							400	
ytñ	gcn	car	tay	ytñ	atg	gay	gay	ttñ	acn	mgn	gay	cnñ	ytñ	tay	ath	1248
leu	ala	gñn	tyr	leu	met	asp	asp	pñe	thr	arg	asp	pro	leu	tyr	ile	
			405					410							415	
ytñ	gay	aay	aay	cay	acn	cay	ytñ	ytñ	gtn	gay	aay	ggn	tgy	cay		1296
leu	asp	asn	asn	his	thr	his	leu	leu	leu	val	asp	asn	gñy	cys	his	
		420						425							430	
ggn	cay	cnñ	gtn	gar	gcn	aar	ytñ	mgn	aay	car	ytñ	gar	aar	tay		1344
gñy	his	pro	thr	val	glu	ala	lys	leu	arg	asn	gñn	leu	glu	lys	tyr	
		435						440							445	
ath	wen	gar	mgn	acn	ath	car	gay	wen	aay	tay	ggn	ggn	aar	ath	cnñ	1392
ile	ser	glu	arg	thr	ile	gñn	asp	ser	asn	tyr	gñy	gñy	lys	ile	pro	
		450						455							460	
ath	gtn	tgy	ttñ	gcn	car	ggn	ggn	ggn	aar	gar	acn	ytñ	aar	gcn	ath	1440
ile	val	cys	pñe	ala	gñn	gñy	gñy	glu	lys	glu	thr	leu	lys	ala	ile	
		465						470							475	
aay	acn	wen	ath	aar	aay	aar	ath	cnñ								

Ytn aar gar ath Ytn gar tgy wsn cay Ytn acn gtn ath aar atg
Leu Lys Glu Ile Leu Glu Cys Ser His Leu Leu Thr Val Ile Lys Met
545 550 555 560
gar gar gcn ggn gar gar ath gtn wsn aay gcn ath wsn tay gcn Ytn
Glu Glu Ala Gly Asp Glu Ile Val Ser Asn Ala Ile Ser Tyr Ala Leu
565 570 575
tay aar gcn tly wsn acn wsn gar car gar aar gar tgy aay ggn
Tyr Lys Ala Phe Ser Thr Ser Glu Glu Asp Lys Asp Asn Trp Asn Gly
580 585 590
car Ytn aar Ytn Ytn gar tgg aay car Ytn gar Ytn gcn aay gar
Glu Leu Lys Leu Leu Glu Trp Asn Glu Leu Asp Leu Ala Asn Asp
595 600 605
gar ath tly acn aay gar ggn tgg gar wsn gcn gar Ytn car gar
Glu Ile Phe Thr Asn Asp Arg Arg Trp Glu Ser Ala Asp Leu Glu
610 615 620
gtn atg tly acn gcn Ytn ath aar gar ggn ccn aar tly gtn gtn Ytn
Val Met Phe Thr Ala Leu Ile Lys Asp Arg Pro Lys Phe Val Arg Leu
625 630 635 640
tly Ytn gar aay ggn Ytn aay Ytn gtn aar tly Ytn acn cay gar gtn
Phe Leu Glu Asn Gly Leu Asn Leu Arg Lys Phe Leu Thr His Asp Val
645 650 655
Ytn acn gar Ytn tly wsn aay cay tly wsn acn Ytn gtn tay gtn aay
Leu Thr Glu Leu Phe Ser Asn His Phe Ser Thr Leu Val Tyr Arg Asn
660 665 670
Ytn car ath gcn aar aay wsn tay aay gar gcn Ytn Ytn tly gtn
Leu Glu Ile Ala Lys Asn Ser Tyr Asn Asp Ala Leu Leu Thr Phe Val
675 680 685
tgg aar Ytn gtn gcn aay tly gtn ggn ggn tly gtn aar gar gar ggn
Trp Lys Leu Val Ala Asn Phe Arg Arg Gly Phe Arg Lys Glu Asp Arg
690 695 700
aay ggn ggn gar gar atg gar ath gar Ytn cay gar gtn wsn ccn ath
Asn Gly Arg Asp Glu Met Asp Ile Glu Leu His Asp Val Ser Pro Ile
705 710 715 720
acn ggn cay ccn Ytn car gcn Ytn tly ath tgg gcn ath Ytn car aay
Thr Arg His Pro Leu Glu Ala Leu Phe Ile Trp Ala Ile Leu Glu Asn
725 730 735
aar aar gar Ytn wsn aar gtn ath tgg gar car acn ggn tgy acn
Lys Lys Glu Leu Ser Lys Val Ile Trp Glu Glu Thr Arg Gly Cys Thr
740 745 750
Ytn gcn gcn Ytn ggn gcn wsn aar Ytn Ytn aar acn Ytn gcn aar gtn
Leu Ala Leu Gly Ala Ser Lys Leu Leu Lys Thr Leu Ala Lys Val
755 760 765
aar aay gar ath aay gcn gcn ggn gar wsn gar gar Ytn gcn aay gar
Lys Asn Asp Ile Asn Ala Ala Gly Glu Ser Glu Glu Leu Ala Asn Glu
770 775 780
tay gar acn ggn gcn gtn gar Ytn tly acn gar tgy cay wsn wsn gar
Tyr Glu Thr Arg Ala Val Glu Leu Phe Thr Glu Cys Tyr Ser Ser Asp
785 790 795 800
gar gar Ytn gcn gar car Ytn Ytn gtn tay wsn tgy gar gcn tgg ggn
Glu Asp Leu Ala Glu Glu Glu Leu Leu Val Tyr Ser Cys Glu Ala Trp Gly
805 810 815 2448

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Gly Ser Asn Cys Leu Glu Leu Ala Val Glu Ala Thr Asp Glu His Phe
820 825 830
ath gcn car ccn ggn gtn car aay tly Ytn wsn aar car tgg tay ggn
Ile Ala Glu Pro Gly Val Glu Asn Phe Leu Ser Lys Glu Trp Tyr Gly
835 840 845
gar ath wsn ggn gar acn aar aay tgg aar ath ath Ytn tgy Ytn tly
Glu Ile Ser Arg Asp Thr Lys Asn Trp Lys Ile Ile Leu Cys Leu Phe
850 855 860
ath ath ccn Ytn gtn ggn tgy ggn tly gtn wsn tly gtn aar ccn
Ile Ile Pro Leu Val Gly Cys Gly Phe Val Ser Phe Arg Lys Lys Pro
865 870 875 880
gtn gar aar cay aar aar Ytn Ytn tgg tay tly gtn gtn tly acn
Val Asp Lys His Lys Lys Leu Leu Trp Tyr Tyr Val Ala Phe Thr
885 890 895
wsn ccn tly gtn gtn tly wsn tgg aay gtn gtn tly tay ath gtn tly
Ser Pro Phe Val Val Phe Ser Trp Asn Val Val Phe Tyr Ile Ala Phe
900 905 910
Ytn Ytn Ytn tly gcn tay gtn Ytn Ytn atg gar tly cay wsn gcn ccn
Leu Leu Leu Phe Ala Tyr Val Leu Leu Met Asp Phe His Ser Val Pro
915 920 925
cay ccn ccn gar Ytn gtn Ytn tay wsn Ytn gtn tly gtn Ytn tly tgy
His Pro Pro Glu Leu Val Leu Tyr Ser Ser Leu Val Phe Val Leu Phe Cys
930 935 940
gar gar gtn ggn car tgg tay gtn aay ggn gtn aay tay tly acn gar
Asp Glu Val Arg Glu Trp Tyr Val Asn Gly Val Asn Tyr Phe Thr Asp
945 950 955 960
Ytn tgg aay gtn atg gar acn Ytn ggn Ytn tly tay tly ath gcn ggn
Leu Trp Asn Val Met Asp Thr Leu Gly Leu Phe Tyr Phe Ile Ala Gly
965 970 975
ath gtn tly gtn Ytn cay wsn wsn aay aar wsn wsn Ytn tay wsn ggn
Ile Val Phe Phe Arg Leu His Ser Ser Asn Lys Ser Ser Tyr Ser Gly
980 985 990
ggn gtn ath tly tgy Ytn gar tay ath ath tly acn Ytn gtn Ytn ath
Arg Val Ile Phe Cys Leu Asp Tyr Ile Ile Phe Thr Leu Arg Leu Ile
995 1000 1005
cay ath tly acn gtn wsn ggn aay Ytn ggn ccn aar ath ath atg Ytn
His Ile Phe Thr Val Ser Arg Asn Leu Gly Pro Lys Ile Ile Met Leu
1010 1015 1020
car ggn atg Ytn ath gar gtn tly tly Ytn Ytn tly gtn gtn
Glu Arg Met Leu Ile Asp Val Phe Phe Phe Leu Phe Ala Val
1025 1030 1035 1040
tgg atg gtn gcn tly ggn gtn gcn ggn car ggn ath Ytn ggn car aay
Trp Met Val Ala Phe Gly Val Ala Arg Arg Glu Glu Asn
1045 1050 1055
gar car ggn tgg ggn tgg ath tly gtn wsn gtn ath tay gar ccn tay
Glu Glu Arg Trp Arg Trp Ile Phe Arg Ser Val Ile Tyr Glu Glu Tyr
1060 1065 1070
Ytn gcn atg tly ggn car gtn ccn wsn gar gtn gtn ggn acn acn tay
Leu Ala Met Phe Gly Glu Val Pro Ser Asp Val Asp Gly Thr Thr Tyr
1075 1080 1085 3264

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Asp Phe Ala His Cys Thr Phe Thr Gly Asn Glu Ser Lys Pro Leu Cys 1100
1090
gtn gar ytn gay gar cay ytn ccn mgn tty ccn gar tgs ath acn 3360
Val Glu Leu Asp Glu His Asn Leu Pro Arg Phe Pro Glu Trp Ile Thr 1115
1105 1110
ath ccn ytn gtn tgy ath tay atg ytn wen acn aay ath ytn ytn gtn 3408
Ile Pro Leu Val Cys Ile Tyr Met Leu Ser Thr Asn Ile Leu Leu Val 1130
1125
aay ytn ytn gtn gcn atg tty ggn tay acn gtn ggn acn gtn car gar 3456
Asn Leu Leu Val Ala Met Phe Gly Tyr Thr Val Gly Thr Val Gln Glu 1140
1145
aay aay gar car gtn tgg aar tty car mgn tay tty ytn gtn car gar 3504
Asn Asn Asp Gln Val Trp Lys Phe Gln Arg Tyr Phe Leu Val Gln Glu 1155
1160
tay tgy wen mgn ytn aay ath ccn tty ccn tty ath gtn tty gcn tay 3552
Tyr Cys Ser Arg Leu Asn Ile Pro Phe Pro Phe Ile Val Phe Ala Tyr 1170
1175
tty tay atg gtn gtn aar aar tgy tty aar tgy tgy aar gar aar 3600
Phe Tyr Met Val Val Lys Lys Cys Phe Lys Cys Cys Lys Glu Lys 1185
1190
aay atg gar wen gtn tgy tgy tty aar aay gar gay aay gar acn 3648
Asn Met Glu Ser Ser Val Cys Cys Phe Lys Asn Glu Asp Asn Glu Thr 1205
1210
ytn gcn tgg gar ggn gtn atg aar gar aay tay ytn gtn aar aay 3696
Leu Ala Trp Glu Gly Val Met Lys Glu Asn Tyr Leu Val Lys Ile Asn 1220
1225
acn aar gcn aay gay acn wen gar gar atg mgn cay mgn tty mgn car 3744
Thr Lys Ala Asn Asp Thr Ser Glu Glu Met Arg His Arg Phe Arg Gln 1235
1240
ytn gay acn aar ytn aay gay ytn aar ggn ytn ytn aar gar ath gcn 3792
Leu Asp Thr Lys Leu Asn Asp Leu Lys Gly Leu Leu Lys Glu Ile Ala 1250
1255
aay aar ath aar 3804
Asn Lys Ile Lys 1265

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ggaagcgag aagtaacaac agatctgggt ccagt atg gca gat cct ggt gat 173
Met Ala Asp Pro Gly Asp 1
5

ggt ccc sgt gca gcg cct ggg gag gtg gct gag ccc cct gga gat gag 221
Gly Pro Arg Ala Ala Pro Gly Glu Val Ala Glu Pro Pro Gly Asp Glu

agt ggt acc tct ggt ggg gag gcc ttc ccc ctc tct tcc ctg gcc aat 20
Ser Gly Thr Ser Gly Gly Glu Ala Phe Pro Leu Ser Ser Leu Ala Asn 30
35
ctg ttt gag ggg gag gaa ggc tcc tct tct tcc ccg gtg gat gct 317
Leu Phe Glu Gly Glu Glu Gly Ser Ser Ser Leu Ser Pro Val Asp Ala 40
45 50
agc cgc cct gct ggc cct ggc gat gga cgt cca aac ctg cgt atg aag 365
Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg Pro Asn Leu Arg Met Lys 55
60 65 70
ttc cag ggc gct ttc cgc aag ggg gtt ccc aac ccc att gac ctg ttg 413
Phe Gln Gly Ala Phe Arg Lys Gly Val Pro Asn Pro Ile Asp Leu Leu 75
80 85
gag tcc acc ctg tac gag tcc tca gta gtg cct ggg ccc aag aaa gcg 461
Glu Ser Thr Leu Tyr Glu Ser Ser Val Val Pro Gly Pro Lys Lys Ala 90
95 100
ccc atg gat tcc ttg ttc gac tac ggc act tac cgt cac cac ccc agt 509
Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr Tyr Arg His His Pro Ser 105
110 115
gac aac aag aga tgg agg aga aag gtc gtg gag aag cag cca cag agc 557
Asp Asn Lys Arg Trp Arg Arg Lys Val Val Glu Lys Gln Pro Gln Ser 120
125 130
ccc aaa gct cct gca ccc cag cca ccc ccc atc ctc aaa gtc ttc aat 605
Pro Lys Ala Pro Ala Pro Gln Pro Pro Pro Ile Leu Lys Val Phe Asn 135
140 145 150
cgg ccc atc ctc ttt gac att gtg tcc cgg ggc tcc act gcg gac cta 653
Arg Pro Ile Leu Phe Asp Ile Val Ser Arg Gly Ser Thr Ala Asp Leu 155
160 165
gat gga ctg ctc tcc ttc ttg acc cac aag aag cgc ctg act gat 701
Asp Gly Leu Leu Ser Phe Leu Leu Thr His Lys Lys Arg Leu Thr Asp 170
175 180
gag gag ttc cgg gag ccg tcc acg ggg aag acc tgc ctg ccc aag gcg 749
Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys Thr Cys Leu Pro Lys Ala 185
190 195
ctg ctg aac cta agc aac ggg cgc aac gac acc atc ccg gtg ttg ctg 797
Leu Leu Asn Leu Ser Asn Gly Arg Asn Asp Thr Ile Pro Val Leu Leu 200
205 210
gac att gcg gag cgc acc ggc aac atg cgt gaa ttc atc aac tgc ccc 845
Asp Ile Ala Glu Arg Thr Gly Asn Met Arg Glu Phe Ile Asn Ser Pro 215
220 225 230
ttc aga gac atc tac tac cga ggc cag aca tcc ctg cac att gcc atc 893
Phe Arg Asp Ile Tyr Tyr Arg Gly Gln Thr Ser Leu His Ile Ala Ile 235
240 245 245
gaa cgg cgc tgc aag cac tac gtg gag ctg ctg gtc cag gga gcc 941
Glu Arg Arg Cys Lys His Tyr Val Glu Leu Leu Val Ala Gln Gly Ala 250
255 260 265
gac gtg cac gcc cag gcc cgc ggc ttc ttc cag ccc aag gat gag 989
Asp Val His Ala Gln Ala Arg Gly Arg Phe Phe Gln Pro Lys Asp Glu 265
270 275
gga ggc tac ttc tac ttt ggg gag ctg ccc ttg tcc ctg gca gcc tgc 1037
Gly Gly Tyr Phe Phe Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys

280	285	290	
acc aac cag ccg cnc atc gtc aac tac ctg aca gag aac cct cac aag Thr Aon Gln Pro His Ile Val Aon Tyr Leu Thr Gln Aon Pro His Lys 295	100	105	1085
aaa gct gac atg aag cga cag gac tcc aag ggg aac acg gtc ctg ccc Lys Ala Asp Met Arg Arg Gln Asp Ser Arg Gly Aon Thr Val Leu His 315	320	325	1133
ggc ctg gtc gcc atc gcc gac aac acc cga gag aac acc aag ttt gtc Ala Leu Val Ala Ile Ala Asp Aon Thr Arg Gln Aon Thr Lys Phe Val 330	335	340	1181
acc aag atg tac gac ctg ctg ctt ctg aag tgt tca cgc ccc ttc ctg Thr Lys Met Tyr Asp Leu Leu Leu Lys Cys Ser Arg Leu Phe Leu 345	350	355	1229
gac agc aac ctg gag aca gtc ctc aac aat gat ggc ctt tgg cct ctc Aop Ser Aon Leu Gln Thr Val Leu Aon Aon Asp Gly Leu Ser Pro Leu 360	365	370	1277
atg atg gct gcc aag aca ggc aag atc ggg gtc ttt cag cac atc atc Met Met Ala Ala Lys Thr Gly Lys Ile Gly Val Phe Gln His Ile Ile 375	380	385	1325
cga cgt gag gtc aca gat gag gac acc cgg cat ctg tct cgc aag ttc Arg Arg Gln Val Thr Asp Gln Aop Thr Arg His Leu Ser Arg Lys Phe 395	400	405	1373
aag gac tgg gcc tat ggg cct gtc tat tct tct ctc tac gac ctc tcc Lys Asp Trp Ala Tyr Gly Pro Val Tyr Ser Ser Leu Tyr Aon Leu Ser 410	415	420	1421
tcc ctg gac aca tgc ggg gag gag gtc tcc gtc ctg gag atc ctg gtc Ser Leu Asp Thr Cys Gly Gln Val Ser Val Leu Gln Ile Leu Val 425	430	435	1469
tac aac agc aag atc gag aac cgc cat gag atg ctg gct gta ggg ccc Tyr Aon Ser Lys Ile Gln Aon Arg His Gln Met Leu Ala Val Gln Pro 440	445	450	1517
atc aac gaa ctg ctg aca gac aag tgg cgt aag ttt ggg gct gtc tcc Ile Aon Gln Leu Leu Arg Asp Lys Trp Arg Lys Phe Gly Ala Val Ser 455	460	465	1565
ctc tac aac aac gtc gtc tcc tat ctg tgt gcc atg gtc atc ttc acc Phe Tyr Ile Aon Val Val Ser Tyr Leu Cys Ala Met Val Ile Phe Thr 475	480	485	1613
ctc acc gcc tac tat cag cca ctg ggg ggc acc cca ccc tac cct tac Leu Thr Ala Tyr Tyr Gln Pro Leu Gln Gly Thr Pro Tyr Pro Tyr 490	495	500	1661
cgg acc aca gtc gac tac ctg aag ctg gct ggc gag gtc atc acc ctg Arg Thr Thr Val Asp Tyr Leu Arg Leu Ala Gly Gln Val Ile Thr Leu 505	510	515	1709
ctc aca gga gtc ctg ttc ttc acc agt atc aaa gac ttg ttc acg Phe Thr Gly Val Leu Phe Phe Thr Ser Ile Lys Asp Leu Phe Thr 520	525	530	1757
aag aac tgc cct gga gtc aat tct ctc ttc gtc gat ggc tcc ttc cag Lys Lys Cys Pro Gly Val Aon Ser Leu Phe Phe Asp Gly Ser Phe Gln 535	540	545	1805
tta ctc tac ttc atc tac tct gtc ctg gtc gtc tct tct ggg ggc ctc Leu Leu Tyr Phe Ile Tyr Ser Val Leu Val Val Ser Ala Ala Leu 550	555	560	1853

555	560	565	
tac ctg gct ggg atc gag gcc tac ctg gtc atg gtc ttt gcc ctg Tyr Leu Ala Gly Ile Gln Ala Tyr Leu Ala Val Met Val Phe Ala Leu 570	575	580	1901
gtc ctg ggc tgg atg aat ggc ctg tac ttc acc cgc ggg ctg aag ctg Val Leu Gly Trp Met Aon Ala Leu Tyr Phe Thr Arg Gly Leu Lys Leu 585	590	595	1949
acc ggg acc tac agc atc atg atc cag aag atc ctc ttc aac gac ctc Thr Gly Thr Tyr Ser Ile Met Ile Gln Lys Ile Leu Phe Lys Asp Leu 600	605	610	1997
ctc cgc ttc ctg ctt gtc ctg ctc ttc acc atg ggc tat gcc tca Phe Arg Phe Leu Leu Val Tyr Leu Leu Phe Met Ile Gly Tyr Ala Ser 615	620	625	2045
ggc ctg gtc acc ctc ctg aat cgg tgc acc aac atg aag gtc tgt gac Ala Leu Val Thr Leu Leu Aon Pro Cys Thr Aon Met Lys Val Cys Asp 635	640	645	2093
gag gac cag agc aac tgc acg gtc ccc acg tat cct ggc tgc cgc gac Gln Asp Gln Ser Aon Cys Thr Val Pro Thr Tyr Pro Ala Cys Arg Asp 650	655	660	2141
agc gag acc ttc acc gcc ttc ctc ctg gac ctc ttc aag ctc acc atc Ser Gln Thr Phe Ser Ala Phe Leu Leu Asp Leu Phe Lys Leu Thr Ile 665	670	675	2189
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ctg aac atg ctt atc gcc ctc atg ggt gag acc gtc ggc cag gtc tcc Leu Aon Met Leu Ile Ala Leu Met Gly Gln Thr Val Gly Gln Val Ser 715	720	725	2333
aag gag agc aag cac atc tgg aag tgg gag tgg gcc acc acc atc ctg Lys Gln Ser Lys His Ile Tyr Lys Thr Gln Thr Ala Thr Ile Leu 730	735	740	2381
gac atc gag cgt tcc ttc cct gtc ttc ctg aag aag gcc ttc cgc tcc Aop Ile Gln Arg Ser Phe Pro Val Phe Leu Arg Lys Ala Phe Arg Ser 745	750	755	2429
gga gag atg gtc act gtc ggc aag agc tca gat ggc act cgc gac cgc Gly Gln Met Val Thr Val Gly Lys Ser Ser Asp Gly Thr Pro Asp Arg 760	765	770	2477
aag tgg tgc ttc aag gtc gag gag gtc aac tgg tct cac tgg aac cag Arg Trp Cys Phe Arg Val Asp Gln Val Aon Trp Ser His Trp Aon Gln 775	780	785	2535
aac ctg ggc atc atc aac gag gac cct ggc aag agt gaa atc tac cag Aon Leu Gly Ile Ile Aon Gln Asp Pro Gly Lys Ser Gln Ile Tyr Gln 795	800	805	2573
tac tat ggc ttc tcc cac acc gtc ggg cgc ctt cgt agg gat cgt tgg Tyr Tyr Gly Phe Ser His Thr Val Gly Arg Leu Arg Arg Asp Arg Trp 810	815	820	2621
tcc tgg gtc gtc ccc cgc gta gtc ggc ctg aac aag aac tca agc gca Ser Ser Val Val Pro Arg Val Val Gln Leu Aon Lys Aon Ser Ser Ala 825	830	835	2669

45/75

835

gat gaa gtg gta ccc ctg gat aac cta ggg aac ccc aac tgt gac 2717
 Asp Glu Val Val Pro Leu Asp Asn Leu Gly Asn Pro Asn Cys Asp 845
 840

ggc cac cag cag ggc tac gct ccc aag tgg agg acg gac gat acc cca 2765
 Gly His Gln Gln Gly Tyr Ala Pro Lys Trp Arg Thr Asp Ala Pro 870
 855

ctg tag gggccgtgcc agagctcgca cagatagtc aggcttgccc ttcgtccca 2821
 Leu *

ccacacattta ggcattttgtc cggctgtcttc cccaccgcga tgggaccttg gaggtagggg 2881
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<400> 14

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 Glu Pro Pro Gly Asp Glu Ser Gly Thr Ser Gly Gly Glu Ala Phe Pro 30
 20
 Leu Ser Ser Leu Ala Asn Leu Phe Glu Gly Glu Gly Ser Ser Ser 45
 35
 Leu Ser Pro Val Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg 60
 50
 Pro Asn Leu Arg Met Lys Phe Gln Gly Ala Phe Arg Lys Gly Val Pro 75
 65
 Asn Pro Ile Asp Leu Leu Glu Ser Thr Leu Tyr Glu Ser Ser Val 95
 85
 Pro Gly Pro Lys Lys Ala Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr 110
 100
 Tyr Arg His His Pro Ser Asp Asn Lys Arg Trp Arg Arg Lys Val Val 125
 115
 Glu Lys Gln Pro Gln Ser Pro Lys Ala Pro Ala Pro Gln Pro Pro Pro 140
 130
 Ile Leu Lys Val Phe Asn Arg Pro Ile Leu Phe Asp Ile Val Ser Arg 155
 145
 Gly Ser Thr Ala Asp Leu Asp Gly Leu Leu Ser Phe Leu Thr His 175
 165
 Lys Lys Arg Leu Thr Asp Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys 190
 180
 Thr Cys Leu Pro Lys Ala Leu Leu Asn Leu Ser Asn Gly Arg Asn Asp 205
 195
 Thr Ile Pro Val Leu Leu Asp Ile Ala Glu Arg Thr Gly Asn Met Arg 220
 210
 Glu Phe Ile Asn Ser Pro Phe Arg Asp Ile Tyr Tyr Arg Gly Gln Thr 235
 225
 Ser Leu His Ile Ala Ile Glu Arg Arg Cys Lys His Tyr Val Glu Leu 255
 245
 Leu Val Ala Gln Gly Ala Asp Val His Ala Gln Ala Arg Gly Arg Phe 270
 260
 Phe Gln Pro Lys Asp Glu Gly Tyr Phe Tyr Phe Gly Glu Leu Pro 285
 275
 Leu Ser Leu Ala Ala Cys Thr Asn Gln Pro His Ile Val Asn Tyr Leu 300
 290
 Thr Glu Asn Pro His Lys Lys Ala Asp Met Arg Arg Gln Asp Ser Arg

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305 Gly Asn Thr Val Leu His Ala Leu Val Ala Ile Ala Asp Asn Thr Arg 315
 325
 Glu Asn Thr Lys Phe Val Thr Lys Met Tyr Asp Leu Leu Leu Lys 335
 340
 Cys Ser Arg Leu Phe Leu Asp Ser Asn Leu Glu Thr Val Leu Asn Asn 350
 355
 Asp Gly Leu Ser Pro Leu Met Met Ala Ala Lys Thr Gly Lys Ile Gly 365
 370
 Val Phe Gln His Ile Ile Arg Arg Glu Val Thr Asp Glu Asp Thr Arg 380
 390
 His Leu Ser Arg Lys Phe Lys Asp Trp Ala Tyr Gly Pro Val Tyr Ser 400
 405
 Ser Leu Tyr Asp Leu Ser Ser Leu Asp Thr Cys Gly Glu Val Ser 415
 420
 Val Leu Glu Ile Leu Val Tyr Asn Ser Lys Ile Glu Asn Arg His Glu 430
 435
 Met Leu Ala Val Glu Pro Ile Asn Glu Leu Leu Arg Asp Lys Trp Arg 445
 450
 Lys Phe Gly Ala Val Ser Phe Tyr Ile Asn Val Val Ser Tyr Leu Cys 460
 465
 Ala Met Val Ile Phe Thr Leu Thr Ala Tyr Tyr Gln Pro Leu Glu Gly 475
 485
 Thr Pro Pro Tyr Pro Tyr Arg Thr Thr Val Asp Tyr Leu Arg Leu Ala 490
 500
 Gly Glu Val Ile Thr Leu Phe Thr Gly Val Leu Phe Phe Thr Ser 510
 515
 Ile Lys Asp Leu Phe Thr Lys Lys Cys Pro Gly Val Asn Ser Leu Phe 525
 530
 Val Asp Gly Ser Phe Gln Leu Leu Tyr Phe Ile Tyr Ser Val Leu Val 540
 545
 Val Val Ser Ala Ala Leu Tyr Leu Ala Gly Ile Glu Ala Tyr Leu Ala 555
 565
 Val Met Val Phe Ala Leu Val Leu Gly Trp Met Asn Ala Leu Tyr Phe 570
 580
 Thr Arg Gly Leu Lys Leu Thr Gly Thr Tyr Ser Ile Met Ile Gln Lys 585
 595
 Ile Leu Phe Lys Asp Leu Phe Arg Phe Leu Leu Val Tyr Leu Leu Phe 605
 610
 Met Ile Gly Tyr Ala Ser Ala Leu Val Thr Leu Leu Asn Pro Cys Thr 620
 625
 Asn Met Lys Val Cys Asp Glu Asp Gln Ser Asn Cys Thr Val Pro Thr 635
 645
 Tyr Pro Ala Cys Arg Asp Ser Glu Thr Phe Ser Ala Phe Leu Leu Asp 655
 660
 Leu Phe Lys Leu Thr Ile Gly Met Gly Asp Leu Glu Met Leu Ser Ser 665
 675
 Ala Lys Tyr Pro Val Val Phe Ile Leu Leu Leu Val Thr Tyr Ile Ile 685
 690
 Leu Thr Phe Val Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu 700
 710
 Thr Val Gly Gln Val Ser Lys Glu Ser Lys His Ile Trp Lys Leu Gln 715
 725
 Trp Ala Thr Thr Ile Leu Asp Ile Glu Arg Ser Phe Pro Val Phe Leu 735
 740
 Arg Lys Ala Phe Arg Ser Gly Glu Met Val Thr Val Gly Lys Ser Ser 745
 755
 Asp Gly Thr Pro Asp Arg Trp Cys Phe Arg Val Asp Glu Val Asn 765
 775
 Trp Ser His Trp Asn Gln Asn Leu Gly Ile Ile Asn Glu Asp Pro Gly 780
 790
 Lys Ser Glu Ile Tyr Gln Tyr Tyr Gly Phe Ser His Thr Val Gly Arg 800
 805
 Leu Arg Arg Asp Arg Trp Ser Ser Val Val Pro Arg Val Val Glu Leu 815
 820
 Asn Lys Asn Ser Ser Ala Asp Glu Val Val Pro Leu Asp Asn Leu 830
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 Gly Asn Pro Asn Cys Asp Gly His Gln Gln Gly Tyr Ala Pro Lys Trp 845

850
Arg Thr Asp Asp Ala Pro Leu
865 870

860

<210> 15

<211> 2613

<212> DNA

<213> Artificial Sequence

<220> CDS

<223> (1)...(2613)

<223> Generic sequence that encompasses all nucleotide
sequences that encode mouse TRPV4 having amino
acid sequence as shown in SEQ ID NO:14

<221> misc. feature

<222> 69,78,102,105,138,141,144,150,165,264,279,282,318,354,402,
477,486,513,567,609,687,723,870,957,1062,1080,1116,1209,
1248,1251,1266,1296,1323,1410,1431,1584,1626,1644,1671,1689,
1809,1890,1950,2001,2061,2064,2178,2187,2241,2274,2301,2304,2358,2406,
2433,2469,2472,2508,2511

<223> n = A,T,C or G if after TC;

n = T or C if after AG

<221> misc. feature

<222> 27,168,192,204,228,342,366,372,375,453,480,537,558,618,657,
672,696,711,744,807,813,945,948,960,1008,1065,1173,1176,1500,1212,
1338,1380,1397,1509,1782,1848,1983,2238,2259,2271,2322,2325,2337,2448

<223> n = A,T,C or G if after CG;

n = A or G if after AG

<221> misc. feature

<222> all "n" not specified above

<223> n = A,T,C or G

<400> 15

atg gcn gay ccn ggn gay ggn ccn mgn gcn ccn ggn gar gcn gcn
Met Ala Asp Pro Gly Asp Gly Pro Arg Ala Ala Pro Gly Glu Val Ala
1 5 10 15

48

gar ccn ccn ggn gay gar wen ggn acn wen ggn ggn gar gcn tly ccn
Glu Pro Pro Gly Asp Glu Ser Gly Thr Ser Gly Gly Glu Ala Phe Pro
20 25 30

96

ytg wen wen ytn gcn aay ytn tly gar ggn gar gar ggn wen wen
Leu Ser Ser Leu Ala Asn Leu Leu Phe Glu Gly Glu Gly Ser Ser Ser
35 40 45

144

ytg wen ccn gtn gay gcn wen mgn ccn gcn ggn ccn ggn gay ggn mgn
Leu Ser Pro Val Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg
50 55 60

192

ccn aay ytn mgn atg aar tly car ggn gcn tly mgn aar ggn gtn ccn
Pro Asn Leu Arg Met Lys Phe Gln Gly Ala Phe Arg Lys Gly Val Pro
65 70 75 80

240

aay ccn ath gay ytn ytn gar wen acn ytn tay gar wen wen gtn gtn
Asn Pro Ile Asp Leu Leu Glu Ser Thr Leu Tyr Glu Ser Ser Val Val
85 90 95

288

ccn ggn ccn aar aar gcn ccn atg gay wen ytn tly gay tay ggn acn
Pro Gly Pro Lys Lys Ala Pro Met Met Ser Ser Leu Phe Asp Tyr Gly Thr
100 105 110

336

tay mgn cay cay ccn wen gay aay aar mgn tay mgn mgn aar gtn gtn
Tyr Arg His His Pro Ser Asp Asn Lys Arg Tyr Arg Lys Val Val
115 120 125

384

gar aar car ccn car wen ccn aar gcn ccn gcn car ccn ccn
Glu Lys Gln Pro Gln Ser Pro Lys Ala Pro Ala Pro Gln Pro Pro
130 135 140

432

ath ytn aar gtn tly aay mgn ccn ath ytn tly gar ath gtn wen mgn
Ile Leu Lys Val Phe Asn Arg Pro Ile Leu Phe Ile Val Ser Arg
145 150 155 160

480

ggn wen acn gcn gay ytn gay ggn ytn wen tly ytn ytn acn cay
Gly Ser Thr Ala Asp Leu Asp Gly Leu Leu Ser Phe Leu Leu Thr His
165 170 175

528

aar aar mgn ytn acn gay gar gar tly mgn gar ccn wen acn ggn aar
Lys Lys Arg Leu Thr Asp Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys
180 185 190

576

acn tgy ytn ccn aar gcn ytn ytn aay ytn wen aay ggn mgn aay gay
Thr Cys Leu Pro Lys Ala Leu Leu Asn Leu Ser Asn Gly Arg Asn Asp
195 200 205

624

acn ath ccn gtn ytn ytn gay ath gcn gar mgn acn ggn aay atg mgn
Thr Ile Pro Val Leu Leu Asp Ile Ala Glu Arg Thr Gly Asn Met Arg
210 215 220

672

gar tly ath aay wen ccn tly mgn gay ath tay tay mgn ggn car acn
Glu Phe Ile Asn Ser Pro Phe Arg Asp Ile Tyr Tyr Arg Gly Gln Thr
225 230 235 240

720

wen ytn cay ath gcn ath gar mgn mgn tgy aay cay tay gtn gar ytn
Ser Leu His Ile Ala Ile Glu Arg Arg Cys Lys His Tyr Val Glu Leu
245 250 255

768

ytg gtn gcn car ggn gcn gay gtn cay gcn car gcn mgn ggn mgn tly
Leu Val Ala Gln Gly Ala Asp Val His Ala Gln Ala Arg Gly Arg Phe
260 265 270

816

tly car ccn aar gay gar ggn ggn tay tly tay tly ggn gar ytn ccn
Phe Gln Pro Lys Asp Glu Gly Tyr Tyr Phe Tyr Phe Gly Glu Leu Pro
275 280 285

864

ytg wen ytn gcn gcn tgy acn aay car ccn cay ath gtn aay tay ytn
Leu Ser Leu Ala Ala Cys Thr Asn Gln Pro His Ile Val Asn Tyr Leu
290 295 300

912

acn gar aay ccn cay aar aar gcn gay atg mgn mgn car gay wen mgn
Thr Glu Asn Pro His Lys Lys Ala Asp Met Arg Arg Gln Asp Ser Arg
305 310 315 320

960

ggn aay acn gtn ytn cay gcn ytn gtn gcn ath gcn gay aay acn mgn
Gly Asn Thr Val Leu His Ala Leu Val Ala Ile Ala Asp Asn Thr Arg
325 330 335

1008

gar aay acn aar tly gtn acn aar atg tay gay ytn ytn ytn aar
Glu Asn Thr Lys Phe Val Thr Lys Met Tyr Asp Leu Leu Leu Lys
340 345 350

1056

tgy wen mgn ytn tly ytn gay wen aay ytn gar acn gtn ytn aay
Cys Ser Arg Leu Leu Phe Leu Asp Ser Asn Leu Glu Thr Val Asn Asn
355 360 365

1104

gay ggn ytn wen ccn ytn atg atg gcn gcn aar acn ggn aar ath ggn
Asp Gly Leu Ser Pro Leu Leu Met Met Ala Ala Lys Thr Gly Lys Ile Gly
370 375 380

1152

atg ggc gat tcc agc gna ggc ccc cgc ggc ggc ggc ggc ggc ggc 48
Met Ala Asp Ser Ser Glu Gly Pro Arg Ala Gly Pro Gly Glu Val Ala 15
1
gag ctc ccc ggc gat gag agt ggc acc cca ggt ggc ggc ggc ttc cct 96
Glu Leu Pro Gly Asp Glu Ser Gly Thr Pro Gly Gly Glu Ala Phe Pro 25
20
ctc tcc tcc ctc ggc aac ctc ttc gag ggc gag gat ggc tcc ctc tgc 144
Leu Ser Ser Leu Ala Asn Leu Phe Glu Gly Glu Asp Gly Ser Leu Ser 40
35
ccc tca cgc gct gat ggc agt cgc cct gct ggc cca ggc gat ggc cga 192
Pro Ser Pro Ala Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg 55
50
cca aat cty cgc atg aag ttc cag ggc ggc ttc cgc aag ggc gtc ccc 240
Pro Asn Leu Arg Met Lys Phe Glu Gly Ala Phe Arg Lys Gly Val Pro 70
65
aac ccc atc gat cty cty gag tcc acc cta tat gag tcc tgc gty gty 288
Asn Pro Ile Asp Leu Leu Glu Ser Thr Leu Tyr Glu Ser Ser Val Val 85
90
cct ggc ccc aag aaa gca ccc atg gac tca cty ttc gac tac ggc acc 336
Pro Gly Pro Lys Lys Ala Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr 100
105
tat cgt cac cac tcc agt gac aac aag aag tgc aag aag aat atc ata 384
Tyr Arg His His Ser Ser Asp Asn Lys Arg Trp Arg Lys Lys Ile Ile 115
120
gag aag cag cgc cag agc ccc aaa ggc cct ggc cct cag cgc ccc ccc 432
Glu Lys Glu Pro Glu Ser Pro Lys Ala Pro Ala Pro Glu Pro Pro Pro 130
135
atc ctc aaa gtc ttc aac cgc cct atc ctc ttc gac atc gty tcc cgc 480
Ile Leu Lys Val Phe Asn Arg Pro Ile Leu Phe Asp Ile Val Ser Arg 145
150
ggc tcc act gct gac cty gac ggc cty ctc cca ttc tgc cty acc cac 528
Gly Ser Thr Ala Asp Leu Asp Gly Leu Leu Pro Phe Leu Leu Thr His 165
170
aag aaa cgc cta act gat gag gag ttc cga gag cca tct acg ggc aag 576
Lys Lys Arg Leu Thr Asp Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys 180
185
acc tgc cty ccc aag ggc ttc cty aac cty agc aat ggc cgc aac gac 624
Thr Cys Leu Pro Lys Ala Leu Leu Asn Leu Ser Asn Gly Arg Asn Asp 195
200
acc atc cct gty cty cty gac atc ggc ggc ggc acc ggc aac atg ggc 672
Thr Ile Pro Val Leu Leu Asp Ile Ala Glu Arg Thr Gly Asn Met Arg 210
215
gag ttc att aac tgc ccc ttc cgt gac atc tac tat cga ggt cag aca 720
Glu Phe Ile Asn Ser Pro Phe Arg Asp Ile Tyr Tyr Arg Gly Glu Thr 225
230
ggc cty cac atc ggc acc gag cgt cgc tgc aaa cac tac gty gaa ctc 768
Ala Leu His Ile Ala Ile Glu Arg Arg Lys His Tyr Val 245
250
ctc gty ggc cag gga gct gat gtc cac ggc cag ggc cgt ggc ggc ttc 816
Leu Val Ala Glu Gly Ala Asp Val His Ala Glu Ala Arg Arg Phe 260
265
270

ttc cag ccc aag gat gag ggc ggc tac ttc tac ttc ggc gag cty ccc 864
Phe Glu Pro Lys Asp Glu Gly Gly Tyr Phe Tyr Phe Gly Glu Leu Pro 275
280
ctg tgc cty gct ggc tgc acc aac cag ccc cac att gtc aac tac cty 912
Leu Ser Leu Ala Ala Cys Thr Asn Glu Pro His Ile Val Asn Tyr Leu 290
295
acc gag aac ccc cac aag aag ggc gat atg cgc ggc cag gac tgc cga 960
Thr Glu Asn Pro His Lys Lys Ala Asp Met Arg Arg Glu Asp Ser Arg 305
310
ggc aac aca gty cty cat ggc cty gty ggc att gct gac aac acc cgt 1008
Gly Asn Thr Val Leu His Ala Leu Val Ala Ile Ala Asp Asn Thr Arg 325
330
gag aac acc aag ttc gtc acc aag atg tac gac cty cty cty cty aag 1056
Glu Asn Thr Lys Phe Val Thr Lys Met Tyr Asp Leu Leu Leu Lys 340
345
tgt ggc cgc ctc ttc ccc gac agc aac cty gag ggc gty ctc aac aac 1104
Cys Ala Arg Leu Leu Phe Pro Asp Ser Asn Leu Glu Ala Val Leu Asn 355
360
gag ggc ctc tgc ccc ctc atg atg gct ggc aag acg ggc aag att ggc 1152
Asp Gly Leu Ser Pro Leu Met Met Ala Ala Lys Thr Gly Lys Ile Gly 370
375
atc ttc cag cac atc atc cgc ggc gag gty acg gat gag gac aca cgc 1200
Ile Phe Glu His Ile Ile Arg Arg Glu Val Thr Asp Glu Asp Thr Arg 385
390
cac cty tcc cgc aag ttc aag gac tgc ggc tat ggc cca gty tat ttc 1248
His Leu Ser Arg Lys Phe Lys Asp Trp Ala Tyr Gly Pro Val Tyr Ser 405
410
tgc ctt tat gac ctc tcc tcc cty gac acg tgt ggc gaa gag ggc tcc 1296
Ser Leu Tyr Asp Leu Ser Ser Leu Asp Thr Cys Gly Glu Glu Ala Ser 420
425
gty cty gag atc cty gty tac aac agc aag att gag aac cgc cac ggc 1344
Val Leu Glu Ile Leu Val Tyr Asn Ser Lys Ile Glu Asn Arg His Glu 435
440
atg cty gct gty gag ccc atc aat gaa cty cty ggc gag aag tgc cgc 1392
Met Leu Ala Val Glu Pro Ile Asn Glu Leu Leu Asp Lys Trp Arg 450
455
aag ttc ggc ggc gtc tcc ttc tac atc aac atc gty gtc tcc tac cty tgc 1440
Lys Phe Gly Ala Val Ser Phe Tyr Ile Asn Val Val Ser Tyr Leu Cys 465
470
ggc atg gtc atc ttc act ctc acc ggc tac tac cag cgc cty gag ggc 1488
Ala Met Val Ile Phe Thr Leu Thr Ala Tyr Tyr Glu Pro Leu Glu Gly 485
490
aca cgc cgc tac cct tac cgc acc acg gty gac tac cty cgc gct gct 1536
Thr Pro Pro Tyr Pro Tyr Arg Thr Thr Val Asp Tyr Leu Leu Ala 500
505
ggc gag gtc att acg cty ttc act ggc gty cty ttc ttc ttc acc aac 1584
Gly Glu Val Ile Thr Leu Phe Thr Gly Val Leu Phe Phe Thr Asn 515
520
atc aaa gac tgc ttc atg aag aaa tgc cct gga gty aat tct ctc ttc 1632
Ile Lys Asp Leu Leu Phe Met Lys Lys Cys Pro Gly Val Asn Ser Leu Phe 530
535
540

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att gat ggc tcc ttc cag ctg ctc tac ttc atc tac tat gtc ctg gtc 1680
ile asp gly ser phe gln leu leu tyr phe ile tyr ser val leu val 560
545
atc gtc tca gca gcc ctc tac ctg gca ggg atc gag gcc tac ctg gcc 1728
ile val ser ala ala leu tyr leu ala gly ile glu ala tyr leu ala 575
565
gtg atg gtc ttt gcc ctg gtc ctg ggc tgg atg aat gcc ctt tac ttc 1776
val met val phe ala leu val leu gly trp met asn ala leu tyr phe 590
580
acc cgt ggg ctg aag ctg acg ggg acc tat agc atc atg atc cag aag 1824
thr arg gly leu lys leu thr gly thr tyr ser ile met ile gln lys 600
595
att ctc ttc aag gac ctt ttc cga ttc ctg ctc gtc tac ttg ctc ttc 1872
ile leu phe lys asp leu phe arg phe leu leu val tyr leu leu phe 610
615
atg atc ggc tac gct tca gcc ctg gtc tcc ctc ctg aac ceg tgt gcc 1920
met ile gly tyr ala ser ala leu val ser leu leu asn pro cys ala 630
625
aac atg aag gtg tgc aat gsg gac cag acc aac tgc aca gtg ccc act 1968
asn met lys val cys asn gln asp gln thr asn cys thr val pro thr 645
650
tac ccc tgg tgc cgt gac agc gag acc ttc agc acc ttc ctc ctg gac 2016
tyr pro ser cys arg asp ser glu thr phe ser thr phe leu leu asp 660
665
ctg ttt aag ctg acc atc ggc atg ggc gac ctg gag atg ctg agc agc 2064
leu phe lys leu thr ile gly met gly asp leu glu met leu ser ser 670
675
acc aag tac ccc gtg gtc ttc atc atc ctg ctg gtc acc tac atc atc 2112
thr lys tyr pro val val phe ile ile leu leu leu thr tyr ile ile 690
695
ctc acc ttt gtg ctg ctc ctc aac atg ctc att gcc ctc atg ggc gag 2160
leu thr phe val leu leu leu asn met leu ile ala leu met gly glu 710
705
aca gtg ggc cag gtc tcc aag gsg agc aag cac atc tgg aag ctg cag 2208
thr val gly gln val ser lys glu ser lys his ile trp lys leu gln 725
730
tgg gcc acc acc atc ctg gac att gag cgc tcc ttc ccc gta ttc ctg 2256
trp ala thr thr ile leu asp ile glu arg ser phe pro val phe leu 740
745
agg aag gcc ttc cgc tct ggg gsg atg gtc acc gtg ggc aag agc tgg 2304
arg lys ala phe arg ser gly glu met val thr val gly lys ser ser 755
760
gac ggc act cct gac cgc agg tgg tgc ttc agg gtg gat gsg gtc aac 2352
asp gly thr pro asp arg arg trp cys phe arg val asp glu val asn 770
775
tgg tct cac tgg aac cag aac ttg ggc atc atc aac gag gac cgc ggc 2400
trp ser his trp asn gln asn leu gly ile ile asn glu asp pro gly 785
790
aag aat gag acc tac cag tat tat ggc ttc tgg cat acc gtg ggc cgc 2448
lys asn glu thr tyr gln tyr tyr gly phe ser his thr val gly arg 805
810

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ctc cgc agg gat cgc tgg tcc ctg gtc gta ccc cgc gtg gaa ctg 2496
leu arg arg asp arg trp ser ser val val pro arg val val glu leu 820
825
aac aag aac ctg aac ccg gac cag gtc gtc gtc cct ctg gac agc atg 2544
asn lys asn ser asn pro asp glu val val pro leu asp ser met 835
840
ggg aac ccc cgc tgc gat ggc cac cag cag ggt tac ccc cgc aag tgg 2592
gly asn pro arg cys asp gly his gln gln gly tyr pro arg lys trp 850
855
agg act gag gac gcc ccg ctc tag 2616
arg thr glu asp ala pro leu * 860
865
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<212> PRT
<213> Homo sapiens
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Glu Leu Pro Gly Asp Glu Ser Gly Thr Pro Gly Gly Glu Ala Phe Pro 15
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Leu Ser Ser Leu Ala Asn Leu Phe Glu Gly Glu Asp Gly Ser Leu Ser 30
35
Pro Ser Pro Ala Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg 45
50
Pro Asn Leu Arg Met Lys Phe Gln Gly Ala Phe Arg Lys Gly Val Pro 60
65
Asn Pro Ile Asp Leu Leu Glu Ser Thr Leu Tyr Glu Ser Ser Val Val 75
80
Pro Gly Pro Lys Lys Ala Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr 90
95
Tyr Arg His His Ser Ser Asp Asn Lys Arg Trp Arg Lys Lys Ile Ile 110
115
Glu Lys Gln Pro Gln Ser Pro Lys Ala Pro Ala Pro Gln Pro Pro Pro 125
130
Ile Leu Lys Val Phe Asn Arg Pro Ile Leu Phe Asp Ile Val Ser Arg 140
145
Gly Ser Thr Ala Asp Leu Asp Gly Leu Leu Pro Phe Leu Leu Thr His 155
160
Lys Lys Arg Leu Thr Asp Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys 170
175
Thr Cys Leu Pro Lys Ala Leu Leu Asn Leu Ser Asn Gly Arg Asn Asp 185
190
Thr Ile Pro Val Leu Leu Asp Ile Ala Glu Arg Thr Gly Asn Met Arg 200
205
Glu Phe Ile Asn Ser Pro Phe Arg Asp Ile Tyr Tyr Arg Gly Gln Thr 210
215
225
Ala Leu His Ile Ala Ile Glu Arg Arg Cys Lys His Tyr Val Glu Leu 230
235
Leu Val Ala Gln Gly Ala Asp Val His Ala Gln Ala Arg Gly Arg Phe 245
250
Phe Gln Pro Lys Asp Glu Gly Tyr Phe Tyr Phe Gly Glu Leu Pro 260
265
Leu Ser Leu Ala Ala Cys Thr Asn Gln Pro His Ile Val Asn Tyr Leu 270
275
Thr Glu Asn Pro His Lys Lys Ala Asp Met Arg Gln Asp Ser Arg 285
290
Gly Asn Thr Val Leu His Ala Leu Val Ala Ile Ala Asp Asn Thr Arg 300
305
Glu Asn Thr Lys Phe Val Thr Lys Met Tyr Asp Leu Leu Leu Lys 310
315
Cys Ala Arg Leu Phe Pro Asp Ser Asn Leu Glu Ala Val Leu Asn Asn 320
325
330
335
340
345
350

355 Asp Gly Leu Ser Pro Leu Met Ala Ala Thr Thr Gly Lys Ile Gly
 370 Ile Phe Gln His Ile Ile Arg Arg Gln Val Thr Asp Gln Asp Thr Arg
 395 His Leu Ser Arg Lys Phe Lys Asp Trp Ala Tyr Gly Pro Val Tyr Ser
 Ser Leu Tyr Asp Leu Ser Ser Leu Asp Thr Cys Gly Gln Gln Ala Ser
 Val Leu Gln Ile Leu Val Tyr Asn Ser Lys Ile Gln Asn Arg His Gln
 435 Met Leu Ala Val Gln Pro Ile Asn Gln Leu Leu Arg Asp Lys Trp Arg
 450 Lys Phe Gly Ala Val Ser Phe Tyr Ile Asn Val Val Ser Tyr Leu Cys
 465 Ala Met Val Ile Phe Thr Leu Thr Ala Tyr Tyr Gln Pro Leu Gln Gly
 485 Thr Pro Pro Tyr Arg Thr Thr Val Asp Tyr Leu Arg Leu Ala
 500 Gly Gln Val Ile Thr Leu Phe Thr Gly Val Leu Phe Phe Thr Asn
 515 Ile Lys Asp Leu Phe Met Lys Lys Cys Pro Gly Val Asn Ser Leu Phe
 530 Ile Asp Gly Ser Phe Gln Leu Leu Tyr Phe Ile Tyr Ser Val Leu Val
 545 Ile Val Ser Ala Ala Leu Tyr Leu Ala Gly Ile Gln Ala Tyr Leu Ala
 565 Val Met Val Phe Ala Leu Val Leu Gly Trp Met Asn Ala Leu Tyr Phe
 580 Thr Arg Gly Leu Lys Leu Thr Gly Thr Tyr Ser Ile Met Ile Gln Lys
 595 Ile Leu Phe Lys Asp Leu Phe Arg Phe Leu Leu Val Tyr Leu Leu Phe
 610 Met Ile Gly Tyr Ala Ser Ala Leu Val Ser Leu Leu Asn Pro Cys Ala
 625 Asn Met Lys Val Cys Asn Gln Asp Gln Thr Asn Cys Thr Val Pro Thr
 645 Tyr Pro Ser Cys Arg Asp Ser Gln Thr Phe Ser Thr Phe Leu Leu Asp
 660 Leu Phe Lys Leu Thr Ile Gly Met Gly Asp Leu Gln Met Leu Ser Ser
 675 Thr Lys Tyr Pro Val Val Phe Ile Ile Leu Leu Val Thr Tyr Ile Ile
 690 Leu Thr Phe Val Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Gln
 705 Thr Val Gly Gln Val Ser Lys Gln Ser Lys His Ile Trp Lys Leu Gln
 725 Trp Ala Thr Thr Ile Leu Asp Ile Gln Arg Ser Phe Pro Val Phe Leu
 740 Arg Lys Ala Phe Arg Ser Gly Gln Met Val Thr Val Gly Lys Ser Ser
 755 Asp Gly Thr Pro Asp Arg Arg Trp Cys Phe Arg Val Asp Gln Val Asn
 770 Trp Ser His Trp Asn Gln Asn Leu Gln Ile Ile Asn Gln Asp Pro Gly
 785 Lys Asn Gln Thr Tyr Gln Tyr Tyr Gly Phe Ser His Thr Val Gly Arg
 805 Leu Arg Arg Asp Arg Trp Ser Ser Val Val Pro Arg Val Val Gln Leu
 820 Asn Lys Asn Ser Asn Pro Asp Gln Val Val Pro Leu Asp Ser Met
 835 Gly Asn Pro Arg Cys Asp Gly His Gln Gln Gly Tyr Pro Arg Lys Trp
 850 Arg Thr Gln Asp Ala Pro Leu
 865

<210> 18

<211> 2613
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 <213> Artificial Sequence
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 <221> CDS
 <222> (1) ... (2613)
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 sequences that encode human TRPV4 having amino
 acid sequence as shown in SEQ ID NO:17
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 n = T or C if after AG
 <221> misc_feature
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 1200,1212,1338,1380,1392,1509,1530,1782,1848,1983,2238,2259,2271,2322,
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 n = A or G if after AG
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 Met Ala Asp Ser Ser Gln Gly Pro Arg Ala Gly Pro Gly Gln Val Ala
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 gar ytn ccn ggn gay gar wsn ggn acn ccn ggn ggn gar gcn tcy ccn
 Gln Leu Pro Gly Asp Gln Ser Gly Thr Pro Gly Gly Gln Ala Phe Pro
 20 25 30
 ytn wsn wsn ytn gcn aay ytn tcy gar ggn gar gar ggn wsn ytn wsn
 Leu Ser Ser Leu Ala Asn Leu Phe Gln Gly Gln Asp Gly Ser Leu Ser
 35 40 45
 ccn wsn ccn gcn gay gcn wsn mgn ccn gcn gcn gcn gcn gcn gcn gcn
 Pro Ser Pro Ala Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg
 50 55 60
 ccn aay ytn mgn atg aar tcy car ggn gcn tcy mgn aar ggn gtn ccn
 Pro Asn Leu Arg Met Lys Phe Gln Gly Ala Phe Arg Lys Gly Val Pro
 65 70 75 80
 aay ccn atg gay ytn ytn gar wsn acn ytn tay gar wsn wsn gtn gtn
 Asn Pro Ile Asp Leu Leu Gln Ser Thr Leu Tyr Gln Ser Ser Val Val
 85 90 95
 ccn gcn ccn aar aar gcn ccn atg gay wsn ytn tcy gay tay ggn acn
 Pro Gly Pro Lys Lys Ala Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr
 100 105 110
 tay mgn gay cay wsn wsn gay aay aar mgn tgg mgn aar aar atg atg
 Tyr Arg His His Ser Ser Asp Asn Lys Arg Trp Arg Lys Lys Ile Ile
 115 120 125
 gar aar car ccn car wsn ccn aar gcn ccn gcn ccn car ccn ccn ccn
 Gln Lys Gln Pro Gln Ser Pro Lys Ala Pro Ala Pro Gln Pro Pro Pro
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130	135	57/75	140	58/75	405	410	415
ath ytn aar gtn tty aay mgn ccn ath ytn tty gay ath gtn wsn mgn ile leu lys val phe asn arg pro ile leu phe asp ile val ser arg 145 150 155 160 165 170 175 180	480						
ggn wsn acn gcn gay ytn gay ggn ytn ccn tty ytn ytn acn cay gly ser thr ala asp leu asp gly leu leu pro phe leu leu thr his 165 170 175 180 185 190 195 200	528						
aar aar mgn ytn acn gay gar gar tty mgn gar ccn wsn acn ggn aar lys lys arg leu leu thr asp glu glu phe arg glu pro ser thr gly lys 180 185 190 195 200 205 210 215	576						
acn tgy ytn ccn aar gcn ytn ytn aay ytn wsn aay ggn mgn aay gay thr cys leu leu pro lys ala leu leu asn leu ser asn gly arg asn asp 195 200 205 210 215 220 225 230	624						
acn ath ccn gtn ytn ytn gay ath gcn gar mgn acn ggn aay atg mgn thr ile pro val leu leu leu asp ile ala glu arg thr gly asn met arg 210 215 220 225 230 235 240 245	672						
gar tty ath aay wsn ccn tty mgn gay ath tay mgn ggn car acn glu phe ile asn ser pro phe arg asp ile tyr arg gly gln thr 225 230 235 240 245 250 255 260	720						
gcn ytn cay ath gcn ath gar mgn mgn tgy aar cay tay gtn gar ytn ala leu his ile ala ile glu arg arg cys lys his tyr val glu leu 260 265 270 275 280 285 290 295	768						
ytn gtn gcn car ggn gcn gay gtn cay gcn car gcn mgn ggn mgn tcy leu val ala gln gly ala asp val his ala gln ala arg gly arg phe 290 295 300 305 310 315 320 325	816						
tty car ccn aar gay gar ggn ggn tay tty tay gtn gar ytn ccn phe gln pro lys asp glu gly tyr phe tyr phe gly glu leu pro 275 280 285 290 295 300 305 310	864						
ytn wsn ytn gcn gcn tgy acn aay car ccn cay ath gtn aay tay ytn leu ser leu ala ala cys thr asn gln pro his ile val asn tyr leu 290 295 300 305 310 315 320 325	912						
acn gar aay ccn cay aar aar gcn gay atg mgn mgn car gay wsn mgn thr glu asn pro his lys lys ala asp met arg arg gln asp ser arg 305 310 315 320 325 330 335 340	960						
ggn aay acn gtn ytn cay gcn ytn gtn ccn ath gcn gay aay acn mgn gly asn thr val leu leu his ala leu val ala ile ala asp asn thr arg 325 330 335 340 345 350 355 360	1008						
gar aay acn aar tty gtn acn aar atg tay gay ytn ytn ytn aar glu asn thr lys phe val thr lys met tyr asp leu leu leu lys 340 345 350 355 360 365 370 375	1056						
tgy acn mgn ytn tty ccn gay wsn aay ytn gar gcn gtn ytn aay aay cys ala arg leu phe pro asp ser asn leu glu ala val leu asn asn 355 360 365 370 375 380 385 390	1104						
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ath tty car cay ath ath mgn mgn gar gtn acn gay gar gay acn mgn his phe gln his ile ile arg arg glu val thr asp glu asp thr arg 385 390 395 400 405 410 415 420	1200						
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aar tgy ggn gcn gtn wsn tty tay ath aay gtn gtn wsn tay ytn tgy lys phe gly ala val ser phe tyr ile asn val val ser tyr leu cys 465 470 475 480 485 490 495 500	1440						
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ath aar gay ytn tty atg aar aar tgy ccn ggn gtn aay wsn ytn tty ile lys asp leu phe met lys lys cys pro gly val asn ser leu phe 530 535 540 545 550 555 560 565	1632						
ath gay ggn wsn tty car ytn ytn tay tty ath tay wsn gtn ytn gtn ile asp gly ser phe gln leu leu tyr phe ile tyr ser val leu val 545 550 555 560 565 570 575 580	1680						
ath gtn wsn gcn gcn ytn tay ytn gcn ggn ath gar gcn tay ytn gcn ile val ser ala ala leu tyr leu ala gly ile glu ala tyr leu ala 565 570 575 580 585 590 595 600	1728						
gtn atg gtn tty gcn ytn gtn ggn tgy atg aay gcn ytn tay tcy val met val phe ala leu val leu gly trp met asn ala leu tyr phe 585 590 595 600 605 610 615 620	1776						
acn mgn ggn ytn aar ytn acn ggn acn tay wsn ath atg ath car aar thr arg gly leu lys leu thr gly thr tyr ser ile met ile gln lys 595 600 605 610 615 620 625 630	1824						
ath ytn tty aar gay ytn tty mgn tty ytn gtn tay ytn ytn tcy ile leu phe lys asp leu phe arg phe leu leu val tyr leu leu phe 610 615 620 625 630 635 640 645	1872						
atg ath ggn tay gcn wsn gcn ytn gtn wsn ytn aay ccn tgy gcn met ile gly tyr ala ser ala leu val ser leu leu asn pro cys ala 625 630 635 640 645 650 655 660	1920						
aay atg aar gtn tgy aay gar gay car acn aay tgy acn gtn ccn acn asn met lys val cys asn glu asp gln thr asn cys thr val pro thr 645 650 655 660 665 670 675 680	1968						
tay ccn wsn tgy mgn gay wsn gar acn tcy wsn acn tty ytn ytn gay tyr pro ser cys arg asp ser glu thr phe ser thr phe leu leu asp 665 670 675 680 685 690 695 700	2016						
ytn tty aar ytn acn ath ggn atg ggn gay ytn gar atg ytn wsn leu phe lys leu thr ile gly met gly asp leu glu met leu ser ser 695 700 705 710 715 720 725 730	2064						

675 680 685
acn aar tay cca gln gln tcy ath ath ytn gln cca tay ath ath 2112
Thr Lys Tyr Pro Val Val Phe Ile Ile Leu Leu Val Thr Tyr Ile Ile
690
ytn acn tcy gln ytn ytn aay atg ytn ath gln ytn atg ggn gar 2160
Leu Thr Phe Val Leu Leu Leu Aon Met Leu Ile Ala Leu Met Gly Glu
705 710 715 720
acn gln ggn car gln wan aar gar wan aar cay ath tgg aar ytn car 2208
Thr Val Gly Gln Val Ser Lys Ser Lys His Ile Trp Lys Leu Gln
725 730 735
tgg gcn acn acn ath ytn gay ath gar mgn wan tcy cca gln tcy ytn 2256
Trp Ala Thr Thr Ile Leu Asp Ile Glu Arg Ser Phe Pro Val Phe Leu
740 745 750
mgn aar gcn tcy mgn wan ggn gar atg gln acn gln ggn aar wan wan 2304
Arg Lys Ala Phe Arg Ser Gly Glu Met Val Thr Val Lys Ser Ser
755 760 765
gay ggn acn cca gay mgn mgn tgg tcy tcy mgn gln gay gar gln aay 2352
Asp Gly Thr Pro Asp Arg Arg Trp Cys Phe Arg Val Asp Glu Val Aon
770 775 780
tgg wan cay tgg aay car aay ytn ggn ath ath aay gar gay cca ggn 2400
Trp Ser His Trp Aon Gln Aon Leu Gly Ile Ile Aon Glu Asp Pro Gly
785 790 795 800
aar aay gar acn tay car tay tay ggn tcy wan cay acn gln ggn mgn 2448
Lys Aon Glu Thr Tyr Gln Tyr Tyr Gly Phe Ser His Thr Val Gly Arg
805 810 815
ytn mgn mgn gay mgn tgg wan wan gln gln cca mgn gln gln gar ytn 2496
Leu Arg Arg Asp Arg Trp Ser Ser Val Val Pro Arg Val Val Glu Leu
820 825 830
aay aar aay wan aay cca gay gar gln gln gln cca ytn gay wan atg 2544
Aon Lys Aon Ser Aon Pro Asp Glu Val Val Pro Leu Asp Ser Met
835 840 845
ggn aay cca mgn tay gay ggn cay car car ggn tay cca mgn aar tgg 2592
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Thr Pro Ser Asn 20
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